PTO 06-2408 HAMT

Japanese Patent
Document No.08-034701

ORGANISM TISSUE STORAGE METHOD

[生物組織保存方法]

Shigematsu, Akiyo ; Suzuki, Satoshi ; Kaburagi, Takayuki ; Sueyoshi, Toru

UNITED STATES PATENT AND TRADEMARK OFFICE
Washington, D.C. Feb 2006

Translated by: Schreiber Translations, Inc.

(19) [Publication Office]

Japan Patent Office (JP)

(12) [Kind of Document]

Unexamined Patent Publication (A)

(11) [Publication Number of Unexamined Application]

Japan Unexamined Patent Publication Hei 08-034701

(43) [Publication Date of Unexamined Application]

1996 (1996) February 6

(43) [Publication Date of Unexamined Application]

1996 (1996) February 6

(54) [Title of Invention]

ORGANISM TISSUE STORAGE METHOD

(51) [International Patent Classification, 6th Edition]

A01N1/02

[Number of Claims]

66

[Form of Application]

FD

[Number of Pages in Document]

9

[Request for Examination]

Not yet requested

(21) [Application Number]

Japan Patent Application Hei 06-323651

(22) [Application Date]

1994 (1994) December 1

(31) [Priority Application Number]

Japan Patent Application Hei 06-023296

(32) [Priority Date]

1994 (1994) January 25

(33) [Priority Country]

Japan (JP)

(31) [Priority Application Number]

Japan Patent Application Hei 06-125741

(32) [Priority Date]

1994 (1994) May 16

(33) [Priority Country]

Japan (JP)

(71) [Applicant]

[Identification Number]

390000675

[Name]

SEITAI KAGAKU KENKYUSHO K.K.

[Address]

340-2 Nauchi, Shirai-machi, Inba-gun, Chiba Prefecture

(72) [Inventor]

[Name]

Shigematsu, Akiyo

[Address]

1-22-11 Nanatsugidai, Shirai-machi, Inba-gun, Chiba Prefecture

(72) [Inventor]

[Name]

Suzuki, Satoshi

[Address]

1-22-11 Nanatsugidai, Shirai-machi, Inba-gun, Chiba Prefecture

(72) [Inventor]

[Name]

Kaburagi, Takayuki

[Address]

145 Takatsuka Nitta, Matsudo City, Chiba Prefecture

(72) [Inventor]

[Name]

Sueyoshi, Toru

[Address]

Public Housing Jobandaira Danchi 1, Section 23, Building 102, 2-25 Jobandaira, Matsudo City, Chiba Prefecture

(57) [Abstract]

[Objective]

In order tissue which configuration does organ of organism, for characteristic and function to be maintained with cell level offer

of the method which is preserved.

[Constitution]

It substitutes blood inside organ, with first saccharide solution which does not cause hemolysis, preventing ice in tissue and the occurrence of crystal of solute furthermore by substituting with second saccharide solution which includes organic solvent, it cools to solution nitrogen temperature, preserves the tissue.

tissue sample is stuck to metal needle assembly and cooling in uniform, thawing is done in uniform and rapid by passing.

[Claim (s)]

[Claim 1]

Infusing perfusion solution to blood vessel which leads to organ of animal, to substitute blood in the aforementioned organ with the aforementioned perfusion solution, the aforementioned organ which is excised from the aforementioned animal to cool part at least, temperature decreasing, in organism tissue storage method which preserves tissue of the aforementioned organ to solution nitrogen temperature,

Infusing saccharide solution of first which substantially does not cause the hemolysis as perfusion solution of first in the aforementioned blood vessel, it substitutes blood of intravascular in the aforementioned organ with saccharide solution of this first,

Next, aqueous solution phase separation it does not do in blood vessel which leads to the aforementioned organ in solution nitrogen temperature and as second perfusion solution, and mixed solution of saccharide and water which substantially do not cause crystal phase separation it does not do in solution nitrogen temperature and infusing second saccharide solution which includes organic solvent which substantially does not cause crystal, It substitutes saccharide solution of the aforementioned first in the aforementioned organ with the aforementioned second saccharide solution, after that it designates that it cools as feature to solution nitrogen temperature, organism tissue storage method.

[Claim 2]

The aforementioned second saccharide solution mannitol is included as the aforementioned saccharide, organism tissue storage method. of Claim 1

[Claim 3]

The aforementioned second saccharide solution inulin is included as the aforementioned saccharide, organism tissue storage method. of Claim 1

[Claim 4]

The aforementioned organic solvent is dimethyl sulfoxide or glycerine, organism tissue storage method. of the Claim 1, 2 or 3

[Claim 5]

temperature of the aforementioned second saccharide solution is 0 deg C or less, organism tissue storage method. of Claims 1 through 4 any

[Claim 6]

With saccharide solution of the aforementioned first from the aforementioned blood substitution afterwards all or predetermined portion of the aforementioned organ is excised from the aforementioned animal, organism tissue storage method. of the Claims 1 through 5 any

[Claim 7]

saccharide solution of the aforementioned first includes glucose, organism tissue storage method. of Claims 1 through 6 any

[Claim 8]

substance where saccharide solution of the aforementioned first has monoamine oxidase inhibition is included, organism tissue storage method. of Claim 1 to 7 any

[Claim 9]

The aforementioned animal is mammal, organism tissue storage method. of Claim 1 to 8 any

[Claim 10]

Infusing perfusion solution to blood vessel which leads to organ of animal, to substitute blood in the aforementioned organ with the aforementioned perfusion solution, the aforementioned organ which is

excised from the aforementioned animal to cool part at least, temperature decreasing, in organism tissue storage method which preserves tissue of the aforementioned organ to solution nitrogen temperature,

Blocking peripheral nerve of region which includes the aforementioned organ,

Infusing isotonic saccharide solution of first which substantially does not cause the hemolysis as perfusion solution of first in the aforementioned blood vessel, it substitutes blood of intravascular in the aforementioned organ with saccharide solution of this first,

Next, aqueous solution phase separation it does not do in blood vessel which leads to the aforementioned organ in solution nitrogen temperature and as second perfusion solution, and mixed solution of saccharide and water which substantially do not cause crystal phase separation it does not do in solution nitrogen temperature and infusing second saccharide solution which includes organic solvent which substantially does not cause crystal, It substitutes saccharide solution of the aforementioned first in the aforementioned organ with the aforementioned second saccharide solution, after that it designates that it cools as feature to solution nitrogen temperature, organism tissue storage method.

[Claim 11]

The aforementioned second saccharide solution mannitol is included as the aforementioned saccharide, organism tissue storage method. of Claim 10

[Claim 12]

The aforementioned organic solvent is dimethyl sulfoxide or glycerine, organism tissue storage method. of the Claim 10 or 11

[Claim 13]

temperature of the aforementioned second saccharide solution is 0 deg C or less, Claim 10 or organism tissue storage method. of 12 any

[Claim 14]

With saccharide solution of the aforementioned first from the aforementioned blood substitution afterwards all or predetermined portion of the aforementioned organ is excised from the aforementioned animal, Claim 10 or organism tissue storage method.

of 13 any

[Claim 15]

saccharide solution of the aforementioned first includes glucose, Claim 10 or organism tissue storage method. of 14 any

[Claim 16]

peripheral nerve is blocked by introducing peripheral nerve shielding agent into intravascular or the intraperitoneal of the aforementioned animal, Claim 10 or organism tissue storage method. of 15 any

[Claim 17]

It is a substance where the aforementioned peripheral nerve shielding agent has monoamine oxidase inhibition, Claim 10 or organism tissue storage method. of 16 any

[Claim 18]

The aforementioned animal is mammal, Claim 10 or organism tissue storage method. of 17 any

[Claim 19]

organ which is excised from animal to cool part at least, in organism tissue storage method which decreasing, preserves temperature to solution nitrogen temperature,

It sticks to needle assembly which consists of multiple metal needle which is locked parallel to metal pedestal description above with at least portion of organ which is excised, as tissue sample, sequence,

It sticks to this needle assembly and it cools the aforementioned sample which it passes gradually with predetermined velocity, furthermore it designates that decreasing, it preserves temperature with solution nitrogen temperature as feature to the solution nitrogen temperature, organism tissue storage method.

[Claim 20]

The aforementioned predetermined velocity is within even each minute 1.3 deg C to - 80 deg C, the organism tissue storage method. of Claim 19

[Claim 21]

organ which is excised from animal to cool part at least, in organism tissue storage method which decreasing, preserves temperature to solution nitrogen temperature,

It sticks to needle assembly which consists of multiple metal needle which is locked parallel to metal pedestal description above with at least portion of organ which is excised, as tissue sample, sequence,

It sticks to this needle assembly and it cools the aforementioned sample which it passes gradually with predetermined velocity, furthermore to solution nitrogen temperature decreasing, it preserves temperature with solution nitrogen temperature,

When ending preservation, in order recovery to do biological activity of the aforementioned tissue sample, temperature of pedestal of the aforementioned needle assembly it designates that it makes 20 deg C or greater during time when it does not exceed 4 min as feature, organism tissue storage method.

[Claim 22]

When ending preservation, temperature of the aforementioned pedestal is designated as 20 deg C or greater within 3 min, organism tissue storage method. of Claim 21

[Claim 23]

Infusing perfusion solution to blood vessel which leads to organ of animal, to substitute blood in the aforementioned organ with the aforementioned perfusion solution, the aforementioned organ which is excised from the aforementioned animal to cool part at least, in organism tissue storage method which decreasing, preserves temperature to solution nitrogen temperature,

Infusing saccharide solution of first which substantially does not cause the hemolysis as perfusion solution of first in the aforementioned blood vessel, it substitutes blood of intravascular in the aforementioned organ with saccharide solution of this first,

Next, aqueous solution phase separation it does not do in blood vessel which leads to the aforementioned organ in solution nitrogen temperature and as second perfusion solution, and mixed solution of saccharide and water which substantially do not cause crystal phase separation it does not do in solution nitrogen temperature and infusing second saccharide solution which includes organic solvent

which substantially does not cause crystal, saccharide solution of the aforementioned first in the aforementioned organ is substituted with the aforementioned second saccharide solution,

It sticks to needle assembly which consists of multiple metal needle which is locked parallel to metal pedestal with at least portion of the aforementioned organ which is excised from the aforementioned animal as tissue sample, the sequence,

It sticks to this needle assembly and it cools tissue sample which it passes, gradually with predetermined velocity, furthermore it designates that decreasing, it preserves temperature with solution nitrogen temperature as feature to solution nitrogen temperature, the organism tissue storage method.

[Claim 24]

The aforementioned organic solvent is dimethyl sulfoxide or glycerine, organism tissue storage method. of the Claim 23

[Claim 25]

The aforementioned second saccharide solution mannitol is included as the aforementioned saccharide, organism tissue storage method. of Claim 23 or 24

[Claim 26]

The aforementioned second saccharide solution inulin is included as the aforementioned saccharide, organism tissue storage method. of Claim 23 or 24

[Claim 27]

saccharide solution of the aforementioned first Claim 23 which includes the mannitol as main saccharide or organism tissue storage method. of 26 any

[Claim 28]

Claim 23 to which saccharide solution of the aforementioned first includes the glucose or organism tissue storage method. of 26 any

[Claim 29]

The aforementioned predetermined velocity to - 80 deg C Claim 23 which is within the even each minute 1.3 deg C or organism tissue

storage method. of 28 any

[Claim 30]

With saccharide solution of the aforementioned first from the aforementioned blood substitution afterwards all or predetermined portion of the aforementioned organ is excised from the aforementioned animal, Claim 23 or organism tissue storage method. of 29 any

[Claim 31]

Infusing saccharide solution of first which substantially does not cause the hemolysis in blood vessel which leads to organ of animal, it substitutes blood in the aforementioned organ with saccharide solution of the aforementioned first,

all or predetermined portion of the aforementioned organ is excised between this substituted or after substituted,

Next, in the aforementioned blood vessel, aqueous solution phase separation it does not do in solution nitrogen temperature and mixed solution of saccharide and water which substantially do not cause crystal phase separation it does not do in the solution nitrogen temperature and it fills second saccharide solution which includes organic solvent which substantially does not cause crystal substitutes saccharide solution of the aforementioned first in the aforementioned organ with the aforementioned second saccharide solution,

It sticks to needle assembly which consists of multiple metal needle which is locked parallel to metal pedestal with at least portion of the aforementioned organ which is substituted with this second saccharide solution, as tissue sample, passes, sticks to the aforementioned needle assembly and it cools the aforementioned sample which it passes, gradually with predetermined velocity, furthermore to the solution nitrogen temperature decreasing, it preserves temperature with solution nitrogen temperature,

When ending preservation, temperature of the aforementioned sample rising above freezing point of the aforementioned second saccharide solution during time when it does not exceed 4 min with at least temperature of portion of the aforementioned needle assembly as 20 deg C or greater, it consists of fact that it pulls out the aforementioned sample from the aforementioned needle assembly, the organism tissue storage method.

[Claim 32]

The aforementioned organic solvent is dimethyl sulfoxide or glycerine, organism tissue storage method. of the Claim 3 1

[Claim 33]

The aforementioned second saccharide solution mannitol is included as the aforementioned saccharide, Claim 3 1 or organism tissue storage method. of 32

[Claim 34]

The aforementioned second saccharide solution inulin is included as the aforementioned saccharide, Claim 3 1 or organism tissue storage method. of 32

[Claim 35]

predetermined velocity of the aforementioned cooling is within even each minute 1.3 deg C to - 80 deg C, organism tissue storage method. of Claim 3 1 through 3 4any

[Claim 36]

saccharide solution of the aforementioned first organism tissue storage method. of Claim 3 1 through 3 5any which includes mannitol as main saccharide

[Claim 37]

organism tissue storage method. of Claim 3 1 through 3 5any to which saccharide solution of the aforementioned first includes glucose

[Claim 38]

Time when it does not exceed the aforementioned 4 min is within3 min, organism tissue storage method. of Claim 3 1 through 37 any

[Claim 39]

When ending preservation, temperature of pedestal of the aforementioned needle assembly is designated as 20 deg C or greater within 3 min, organism tissue storage method. of the Claim 38

[Claim 40]

Infusing saccharide solution of first which substantially does not cause the hemolysis in blood vessel which leads to organ of animal, it substitutes blood in the aforementioned organ with saccharide solution of the aforementioned first,

Next, in the aforementioned blood vessel, aqueous solution phase separation it does not do in solution nitrogen temperature and mixed solution of saccharide and water which substantially do not cause crystal phase separation it does not do in the solution nitrogen temperature and it fills second saccharide solution which includes organic solvent which substantially does not cause crystal substitutes saccharide solution of the aforementioned first in the aforementioned organ with the aforementioned second saccharide solution,

all or predetermined portion of the aforementioned organ which is substituted with this second saccharide solution is excised from the aforementioned animal,

With this as tissue sample, it sticks to needle assembly which consists of the multiple metal needle which is locked parallel to metal pedestal sequence,

It sticks to the aforementioned needle assembly and it cools the aforementioned sample which it passes, gradually with predetermined velocity, furthermore to the solution nitrogen temperature decreasing, it preserves temperature with solution nitrogen temperature,

When ending preservation, temperature of the aforementioned sample rising above freezing point of the aforementioned second saccharide solution inside predetermined time with at least temperature of portion of the aforementioned needle assembly as 20 deg C or greater,

It consists of fact that the aforementioned sample is pulled out from the aforementioned needle assembly, organism tissue storage method.

[Claim 41]

The aforementioned organic solvent is dimethyl sulfoxide or glycerine, organism tissue storage method. of the Claim 40

[Claim 42]

The aforementioned second saccharide solution mannitol is included

as the aforementioned saccharide, Claim 40 or organism tissue storage method. of 41

[Claim 43]

temperature of the aforementioned second saccharide solution is designated as 0 deg C or less, Claim 40 or organism tissue storage method. of 41

[Claim 44]

saccharide solution of the aforementioned first it is something which substantially does not cause crystal in solution nitrogen temperature, Claim 40 or the organism tissue storage method. of 43 any

[Claim 45]

saccharide solution of the aforementioned first organism tissue storage method. of Claim 44 which includes mannitol as main saccharide

[Claim 46]

organism tissue storage method. of Claim 44 to which saccharide solution of the aforementioned first includes glucose

[Claim 47]

predetermined velocity of the aforementioned cooling is within even each minute 1.3 deg C to - 80 deg C, Claim 40 or organism tissue storage method. of 46

[Claim 48]

When ending preservation, temperature of pedestal of the aforementioned needle assembly is designated as 20 deg C or greater within 3 min, Claim 40 or organism tissue storage method. of 47 any

[Claim 49]

In blood vessel which leads to organ of animal, infusing saccharide solution of first which substantially does not cause hemolysis, it substitutes blood in the aforementioned organ with saccharide solution of the aforementioned first,

Next, in the aforementioned blood vessel, aqueous solution phase

separation it does not do in solution nitrogen temperature and mixed solution of saccharide and water which substantially do not cause crystal phase separation it does not do in the solution nitrogen temperature and it fills second saccharide solution which includes organic solvent which substantially does not cause crystal substitutes saccharide solution of the aforementioned first in the aforementioned organ with the aforementioned second saccharide solution,

It sticks to needle assembly which consists of multiple metal needle which is locked parallel to metal pedestal with all or predetermined portion of the aforementioned organ which is substituted with this second saccharide solution, as the tissue sample, sequence,

It sticks to the aforementioned needle assembly and it cools the aforementioned sample which it passes, gradually with predetermined velocity, furthermore to the solution nitrogen temperature decreasing, it preserves temperature with solution nitrogen temperature,

When ending preservation, inside predetermined time temperature of the aforementioned sample rising above freezing point of the aforementioned second saccharide solution,

It consists of fact that the aforementioned sample is pulled out from the aforementioned needle assembly, organism tissue storage method.

[Claim 50]

The aforementioned organic solvent is dimethyl sulfoxide or glycerine, organism tissue storage method. of the Claim 49

[Claim 51]

The aforementioned second saccharide solution mannitol is included as the aforementioned saccharide, Claim 49 or organism tissue storage method. of 50

[Claim 52]

predetermined velocity of the aforementioned cooling is within even each minute 1.3 deg C to - 80 deg C, Claim 49 or organism tissue storage method. of 51 any

[Claim 53]

Claim 4 9 to which saccharide solution of the aforementioned first includes the glucose or organism tissue storage method. of 52 any

[Claim 54]

When ending preservation, within 4 min temperature of the aforementioned sample it rises - 5 deg C or greater 5 deg C or less, Claim 49 or organism tissue storage method. of 53 any

[Claim 55]

When ending preservation, temperature of the aforementioned sample, within 4 min rising - 5 deg C or greater 5 deg C or less, at ratio below even each minute 4 deg C it rises in 25 deg C or greater, organism tissue storage method. of Claim 54

[Claim 56]

Infusing perfusion solution to blood vessel which leads to organ of animal, to substitute blood in the aforementioned organ with the aforementioned perfusion solution, the aforementioned organ which is excised from the aforementioned animal to cool part at least, temperature decreasing, in organism tissue storage method which preserves at least portion of tissue of the aforementioned organ to solution nitrogen temperature,

Before infusing the aforementioned perfusion solution, blocking peripheral nerve of the region which beforehand includes the aforementioned organ of the aforementioned animal,

Infusing saccharide solution of first which substantially does not cause the hemolysis in blood vessel which leads to the aforementioned organ, it substitutes blood in the aforementioned organ with saccharide solution of the aforementioned first, during this substituting or after that excises the aforementioned organ from the aforementioned animal,

Next, in blood vessel which leads to the aforementioned organ, aqueous solution phase separation it does not do in solution nitrogen temperature and mixed solution of saccharide and water which substantially do not cause crystal phase separation it does not do in solution nitrogen temperature and it fills second saccharide solution which includes the organic solvent which substantially does not cause crystal substitutes the saccharide solution of the aforementioned first in the aforementioned organ with the aforementioned second saccharide solution,

It sticks to needle assembly which consists of multiple metal needle which is locked parallel to metal pedestal with at least portion of the aforementioned organ which is substituted with this second saccharide solution, as tissue sample, passes, sticks to the aforementioned needle assembly and it cools the aforementioned sample which it passes, gradually with predetermined velocity, furthermore to the solution nitrogen temperature decreasing, it preserves temperature with solution nitrogen temperature,

When ending preservation, during time when it does not exceed 4 min temperature of the aforementioned sample rising above freezing point of the aforementioned second saccharide solution,

It consists of fact that the aforementioned sample is pulled out from the aforementioned needle assembly, organism tissue storage method.

[Claim 57]

The aforementioned organic solvent is dimethyl sulfoxide or glycerine, organism tissue storage method. of the Claim 56

[Claim 58]

The aforementioned second saccharide solution mannitol is included as the aforementioned saccharide, Claim 56 or organism tissue storage method. of 57

[Claim 59]

Claim 56 to which saccharide solution of the aforementioned first includes the glucose or organism tissue storage method. of 58 any

[Claim 60]

predetermined velocity of the aforementioned cooling is within even each minute 1.3 deg C to - 80 deg C, Claim 56 or organism tissue storage method. of 59 any

[Claim 61]

When ending preservation, within 4 min temperature of the aforementioned sample it rises - 5 deg C or greater 5 deg C or less, Claim 56 or organism tissue storage method. of 60 any

[Claim 62]

When ending preservation, temperature of the aforementioned sample, within 4 min rising - 5 deg C or greater 5 deg C or less, at ratio below even each minute 4 deg C it rises in 25 deg C or greater, organism tissue storage method. of Claim 61

[Claim 63]

organ which is excised from animal cooling part at least, the temperature decreasing to solution nitrogen temperature and after retaining, thawing doing the aforementioned tissue sample biological activity regarding to organism tissue preservation regeneration method which recovers,

Description above it sticks all or predetermined portion of organ which is excised, to needle assembly which consists of metal needle of plural which is locked parallel to metal pedestal passes and cools, it preserves with solution nitrogen temperature as tissue sample,

When ending preservation, in order recovery to do biological activity of the aforementioned tissue sample, the aforementioned metal needle heating one end at least, thawing it does the aforementioned tissue sample within 4 min with temperature of the aforementioned tissue sample as -5 deg C or greater 5 deg C or less,

From the aforementioned needle assembly the aforementioned tissue sample pulling out,

temperature of thawing backward description tissue sample at ratio below the even each minute 4 deg C rising in 25 deg C or greater, biological activity of the aforementioned tissue sample it designates that it recovers as feature, organism tissue preservation regeneration method.

[Claim 64]

Infusing perfusion solution to blood vessel which leads to organ of animal, after it substitutes blood in the aforementioned organ with the aforementioned perfusion solution, the aforementioned organ which is excised from the aforementioned animal it cools part at least, temperature decreases to solution nitrogen temperature and retaining tissue of the aforementioned organ, thawing doing the aforementioned tissue sample, biological activity regarding to the organism tissue preservation regeneration method which recovers,

Description above it sticks all or predetermined portion of organ which is excised, to needle assembly which consists of metal needle

of plural which is locked parallel to metal pedestal passes and cools, it preserves with solution nitrogen temperature as tissue sample,

When ending preservation, the aforementioned metal needle heating one end at least, thawing it does the aforementioned tissue sample within 4 min with temperature of the aforementioned tissue sample as -5 deg C or greater 5 deg C or less,

From the aforementioned needle assembly the aforementioned tissue sample pulling out,

After thawing 5 minute passage doing at least, temperature of the aforementioned tissue sample rising in 25 deg C or greater, biological activity of the aforementioned tissue sample it designates that it recovers as feature, the organism tissue preservation regeneration method.

[Claim 65]

temperature after thawing while rising, biological activity of the aforementioned tissue sample it recovers to 25 deg C by perfusion doing the buffer which includes saccharide and blood serum protein in blood vessel which leads to the aforementioned tissue sample, organism tissue preservation regeneration method. of Claim 6 4

[Claim 66]

The aforementioned buffer includes dibutyryl cyclic AMP, organism tissue preservation regeneration method. of Claim 6 5

[Description of the Invention]

[0041]

Working Example 3

Putting, you supplied liver which finishes perfusion with the third perfusion solution in box of temperature 37 deg C, relative humidity 100%, filled [2 -^{14}C] diazepam of 185 kBq from portal vein cannulation, you supplied metabolism experimental perfusion solution of below-mentioned composition with flow of 1 ml/min making use of metering pump immediately after that.

Simultaneously same solution was supplied with flow of 1.5 ml/min via artery cannulation.

It continued 20 minute perfusion, it received perfusion solution which at time of the this flows out from liver to funnel, in every 2 min recovered in test tube.

[0042]

10 min centrifugal separation it did perfusion solution which recovers with 3000 revolutions, due to thin layer chromatography, it separated radioactivity metabolite in supernatant, it did detection analysis with radioluminography.

As a result, 4'-hydroxydiazepam, nordazepam, temazepam, oxazepam which is metabolite to other than diazepam which is a parent compound was detected in eluting solution.

As for these, when same experiment was done freezing making use of the liver which is not preserved with metabolite which is recognized, the fact that liver tissue which is preserved by this method has metabolic activity has been shown.

[0043]

In addition, when lactic acid dehydrogenase (LDH) is done quantification, each eluting solution with approximately 100 IU/liter, normal value was shown via perfusion of 20 minute.

This, passing by process of freezing, thawing, shows fact that liver tissue has not received injury.

[0001]

[Field of Industrial Application]

As for this invention it is a low temperature storage method of organism tissue which can do characteristic of cell which configuration does organism tissue storage method, especially tissue and to maintain performance, and something regarding preservation regeneration method.

[0002]

[Prior Art]

In order bacteria or other microorganism and ovum, sperm, cultured cell or other cell, activity 50% or more to be maintained, because it preserves, it cools cell gradually with the speed approximately of each minute 1 deg C, preserves with approximately - 200 deg C, at

time of preservation end method quickly it melts (thawing) is known.

this occasion in order to prevent destruction with freezing of the cell, cell is put in 10% glycerine or in 5 - 10% dimethyl sulfoxide aqueous solution.

[0003]

[Problems to be Solved by the Invention]

microorganism, ovum, sperm or other individual cell long term storage is possible relatively with the above-mentioned method.

But when solution a this way in organ, for example liver, kidney or other organ of animal is filled, characteristic of cell of tissue which is preserved and loss of function were considerable.

[0004]

objective of this invention in order tissue which configuration does the organ of organism, for characteristic and function to be maintained with cell level is to offer method which is preserved.

In addition objective of this invention in order tissue which configuration does organ of organism, for characteristic and function to be maintained with cell level after retaining, biological activity is to offer the method which recovers.

[0005]

[Means to Solve the Problems]

saccharide where organism tissue storage method of this invention in order to achieve this objective, infusing saccharide solution of first which substantially does not cause the hemolysis in blood vessel which leads to predetermined organ of animal, substitutes blood in organ with saccharide solution of first, aqueous solution the phase separation does not do in solution nitrogen temperature and substantially does not cause crystal and, mixed solution of water phase separation it does not do in solution nitrogen temperature and it fills second saccharide solution which includes organic solvent which substantially does not cause crystal to blood vessel, it substitutes saccharide solution of the first which is in intravascular with second saccharide solution, excises all or the predetermined portion of organ, When this it sticks sample of organ which is excised to needle assembly which consists of multiple metal needle which is locked parallel passes, cools gradually with

predetermined velocity, furthermore to solution nitrogen temperature decreasing, it sticks temperature to needle assembly and it preserves sample which it passes with solution nitrogen temperature, ending preservation quickly with at least the temperature of portion of needle assembly as 20 deg C or greater, temperature of sample rising rapidly to above freezing point of second saccharide solution, it consists of fact that it pulls out sample from needle assembly.

organ or other organ of animal furthermore, it can apply this invention to also muscle or other connective tissue.

[0006]

Before perfusion or between perfusion it is possible instead of with first and second saccharide solution of blood excising tissue sample after the substitution (Below, you call perfusion), to excise all or predetermined portion of organ from animal.

predetermined portion, it is a one or a two of liver leaf in for example liver.

To do perfusion or excision, it is necessary to do surgery of the animal.

After excising when perfusion is done, furthermore it is possible to excise portion of tissue after perfusion as sample.

[0007]

Before administering surgery, it is desirable for keeping tissue function to block, peripheral nerve of organ vicinity which becomes, the especially object of animal.

It can achieve shielding of peripheral nerve of object organ vicinity, substance which possesses monoamine oxidase (Below, MAO and brief description) inhibition by infusing to intravascular or intraperitoneal.

There is a for example N- amino alkyl phenothiazine compound, N-amino alkyl dibenzo aza cycloheptadiene compound, isonicotinic acid hydrazide compound, propargyl amine derivative in MAO inhibitor which can be applied to this invention.

It is a for example chlopromazine, promethazine, imipramine, iproniazide, pargyline, etc.

It is possible to add MAO inhibitor also in perfusion solution.

[0008]

perfusion usually, sticks indwelling needle (hollow) to blood vessel which leads to organ and is packed and does.

flow is chosen according to types, thickness of types, blood vessel of organ.

It substitutes with first and second saccharide solution, in order for saccharide solution of respective blood or first not to remain substantially, the fully.

[0009]

saccharide which is included in second saccharide solution, for example, mannitol (mannitol) and like the inulin, phase separation does not do in solution nitrogen temperature and, it must be a kind of saccharide which substantially does not cause crystal.

Substantially crystal is not caused with, means fact that the structure of tissue after retaining or crystal of extent of giving injury to biological activity are not caused.

this saccharide does not have necessity to permeate to intracellular, those which rather are difficult to permeate to intracellular are desirable.

[0010]

As for saccharide solution of first, it is desirable to include kind of saccharide, for example glucose which is useful to maintenance of biological activity of tissue.

concentration of glucose is equal to glucose concentration in tissue, it is desirable or be as close as possible to make concentration.

As for saccharide solution of first, it is necessary to make composition which does not cause hemolysis which becomes cause of remains of the blood cell component to in tissue.

Substantially hemolysis is not caused with, means structure of the tissue and fact that it is not injury of biological activity with the hemolysis.

[0011]

saccharide solution of first and second substantially being a isotonic (isotonic) with respect to the tissue is desirable, if, but blood and lymphatic solution 1/2 or 2 -fold a osmotic pressure of range it should have been.

first and second saccharide solution respectively may include other salts, acid, base, buffer, surfactant, thickener, etc., of sugar.

As for temperature of saccharide solution of first and second which is used for the perfusion, in order to prevent change of tissue, it is desirable to maintain at temperature (Usually, - 10 deg C or + 5 deg C) whose or more of freezing point of solution slow if possible.

[0012]

organic solvent which is used for second saccharide solution mixed solution of water phase separation does not do in solution nitrogen temperature and it must be something which substantially does not cause crystal.

Substantially crystal is not caused with, means fact that the structure of tissue after retaining or crystal of extent of giving injury to biological activity are not caused.

organic solvent which has property a this way is for example glycerine, dimethyl sulfoxide.

concentration of organic solvent in second saccharide solution 5 - 10% is suitable, but to be somewhat lower than this (for example 3%) or to be somewhat high (for example, 15%), it is good.

[0013]

As for metal needle or metal pedestal which are used for needle assembly, surface, does not produce effect on tissue, sugar solution at least, those which consist of metal which in addition does not cause rust and corrosion with those are desirable.

for example stainless steel, gold, platinum, gold plating, chromium plating (Such as iron, brass), etc., is used.

[0014]

Although thickness of metal needle, it sticks tissue sample and passes it is necessary to make thickness which gives sufficient

mechanical strength.

Extent where metal needle is thick, extent and heat conduction where in addition interval is narrow being rapid, when ending preservation with low temperature, temperature of tissue sample it can rise quickly, but the mechanical injury of tissue sample entirety becomes large.

As for thickness of metal needle as for 0.2 or 1.6 mm, interval (Between center) 0.5 or 4 mm are suitable.

[0015]

It sticks to metal needle and as for cooling tissue sample which it passes, at least it is desirable to do to - 80 deg C gradually, for example temperature drop it is desirable to - 80 deg C to make within even each minute 1.3 deg C.

If storing for example sample to cold chest of suitable size, you put temperature drop a this way, in freezer of - 80 deg C it can actualize.

Making use of freezer which program control is done it is good.

[0016]

When ending preservation, rising quickly to temperature (Usually - 5 deg C or + 5 deg C, for example 4 deg C) of melting point or higher of second saccharide solution, thawing it does temperature of sample needle assembly at least by designating temperature of part quickly (Within for example 2 min) as 20 deg C or greater.

Concretely 20 deg C of suitable amount or minute warm water (It is good even with solution other than water) of 40 deg C are let flow in surroundings of for example metal pedestal.

Or it sticks to metal needle assembly and it lets flow perfusion solution (saccharide solution) of suitable temperature around sample which passes.

With method a this way, temperature of tissue sample, within preferably 4 min, rising above freezing point of second saccharide solution, thawing it does.

[0017]

After sample melts, with suitable means, it sticks to base of the

for example metal needle and it pulls out tissue sample from needle assembly by pulling out the fabric and synthetic paper which it passes.

perfusion it does tissue sample which it pulls out with physiological saline, saccharide solution, tissue culture solution, other things of suitable temperature, for example 4 deg C, recovering, it is offered biological activity which is needed at least to business from now on that.

[0018]

While in order to recover, perfusion doing biological activity with solution (Same solution as saccharide solution of for example first)of suitable composition, from temperature which is necessary for thawing of the tissue spending time above minimum limit on approximately 25 deg C, the temperature rising, furthermore it rises to 37 deg C vicinity.

Time of minimum limit with 5 min, 10 min or greater applying, it is desirable to increase temperature to 25 deg C.

From temperature 25 deg C there is not restriction of especially time in rise to37 deg C vicinity.

It is be possible to include dibutyryl cyclic AMP to perfusion solution which is used with this step, useful in prevention of plasma membrane injury under low oxygen concentration.

In addition in perfusion solution it is possible to include hydrocortizone.

[0019]

this invention, is useful, in liver, kidney, pancreas, spleen, testes, ovary, adrenal, brain, thyroid or other low temperature storage of especially mammal.

It is a especially effective in low temperature storage of liver.

[0020]

[Working Principle]

Infusing saccharide solution of first to blood vessel which leads to organ of animal, blood in organ is removed by substituting the blood with saccharide solution of first.

Furthermore infusing second saccharide solution to blood vessel, saccharide solution of first is removed by substituting saccharide solution of first with second saccharide solution, the intravascular is filled up with second saccharide solution.

At time of this, dong way also blood vessel, cell gap is filled up with second saccharide solution.

[0021]

When it sticks tissue sample it is excised (It is possible to excise before or during perfusion), to needle assembly which consists of multiple metal needle which is locked parallel to metal pedestal passes, cools gradually, it sticks and temperature of tissue sample entirety is maintained relatively at uniform by heat conduction which minds metal needle which passes.

It sticks to needle assembly and furthermore by decreasing, maintaining the temperature at this temperature to solution nitrogen temperature, tissue sample which passes is preserved with solution nitrogen temperature.

[0022]

second saccharide solution which from blood vessel is introduced into tissue aqueous solution phase separation not to do in solution nitrogen temperature because and saccharide which substantially does not cause crystal is included, even with the solution nitrogen temperature crystal of saccharide itself or ice, does not occur at least in outside (intravascular, dong way blood vessel, cell gap is included.) of cell of tissue.

In addition second saccharide solution mixes with water well, mixed solution of the water phase separation does not do in solution nitrogen temperature and includes organic solvent which substantially does not cause crystal.

this organic solvent permeates to also intracellular.

Both outside intracellular because this organic solvent exists, ice or the solvent (Or eutectic body) crystal do not occur in tissue in solution nitrogen temperature.

Therefore, destruction of cell is prevented with formation of the ice and solvent crystal.

[0023]

When ending preservation, when at least temperature of portion of needle assembly it is designated quickly as 20 deg C or greater, temperature of tissue sample rises to melting point or higher of second saccharide solution, when portion which touches with metal needle melts, to pull out from needle assembly it is possible sample.

Furthermore when temperature of tissue it rises in 25 deg C or greater, the tissue recovers biological activity.

When metal needle assembly is not used sticking it is through metal needle assembly which passes with heat conduction, comparing, rises rapidly relatively temperature of tissue sample entirety and in uniform.

this quickly of sample entirety and because of uniform temperature rise, as for characteristic or function of tissues and cells there are not times when it is impaired, they can recover after that with adjustment of environment (temperature etc).

[0024]

[Working Example (s)]

It shows Working Example below, makes furthermore exemplary explanation of the this invention.

[実施例 1]			]	
[Working Example 1]			]	
(1)	金属	属針集	集合体の製	製作
(1)	metal	needle collection	assembly	Production

属針集
needle collection

Those which excluded connecting part to shooting tube.) In pedestal of low melting point alloy vertical 10 lines, side 12 line it stood upright (end in upward) with 1 mm interval.

In order to remove tissue sample which it inserts, glass rod of thickness 0.6mm was inserted in lattice between needle alongside pedestal.

[0025]

(2) liver perfusion

open abdominal surgery was administered to healthy rat, indwelling needle was inserted in liver portal vein.

In accordance with conventional method of liver perfusion, in indwelling needle from polyethylene capillary which hitch is done, it filled first perfusion solution of the below-mentioned composition which is made temperature 4 deg C to liver portal vein with the flow of each minute 4 ml, 5 min perfusion did.

D・マンニット	1 g
D- mannitol	1 g
Krebs-Ringer緩衝液	２０ミリリットル
Krebs-Ringer buffer	20 ml

D・マンニット	1 g
D- mannitol	1 g
Krebs-Ringer緩衝液	２０ミリリットル
Krebs-Ringer buffer	20 ml

[0026]

(3) low temperature storage

Immediately after perfusion, avulsion it did liver, stuck to the metal needle assembly and passed.

After storing this to polystyrene foam cold chest, you supplied in freezer of - 80 deg C, (It is cooled slowly within each minute 1 deg C).

3 hours or more passage later, pouring solution nitrogen of suitable amount to cold chest, furthermore it cooled.

While adding solution nitrogen, 3 week it preserved liver sample.

[0027]

thawing of (4) tissue

net sheet was locked in location of depth 2 millimeter of constant temperature water tank of the temperature 25 deg C.

It removed liver sample which it passes to metal needle from cold chest that way, placed on this net sheet.

It is heated with constant temperature water of 25 deg C where metal pedestal flows to surroundings.

It removed 2 min, later extrusion doing liver sample making use of the lattice of glass rod which is inserted on pedestal side of metal needle, it removed from needle.

In liver which you remove from portal vein infusing first perfusion solution of the temperature 4 deg C with flow of each minute 4 ml, 5 min perfusion it did, substituted the second perfusion solution in liver with first perfusion solution.

[0028]

Verification of (5) tissue preservation

stain it did formalin after locking liver sample, to cutting of thickness 5micron.

Result of microscopic observation, cell of liver was preserved well, could recognize especially glycogen granule clearly.

[0029]

		[実施例２]

		[Working Example 2]	
(1)	組織	織採取および潅	潅流
(1)	tissue	recovery and	perfusion

[実施例２]

[Working Example 2]

織採取および潅

recovery and

injection doing 1 ml /weight 100g, blocking peripheral nerve of abdomen, injection doing somno-pentyl of weight per 100 g 0.04mg, narcotic after doing, it administered the open abdominal surgery, polyethylene capillary and it inserted indwelling needle which hitch is done in liver portal vein.

In accordance with conventional method of liver perfusion, in indwelling needle from polyethylene capillary which hitch is done, it filled first perfusion solution of the below-mentioned composition which - designates temperature as 5 deg C to the liver portal vein with flow of each minute 0.9ml, 4 min perfusion did.

While perfusion doing, it excised liver from rat with the fixed method.

D - グルコース			2	g

D- glucose			2	g
BSA			4.5	g
BSA			4. 5	g
酵素阻害剤Pefabloc(Merck)			1	mM相当
enzyme inhibitor Pefabloc (Merck)			1	mM equivalent
p-APMSF			0.1	mM相当
p-APMSF			0. 1	mM equivalent

ide)				
ide)				
Krebs-Henseleit	緩衝液	100 ミリリットル (pH 7.4)		
Krebs-Henseleit	buffer	100 ml (pH 7.4)		
クロロブロマジン		1 g		
chlorobromazine		1 g		
ヘパリン		0.5 ミリリットル		
heparin		0.5 ml		

100 ミリリットル (pH 7.4)

100 ml (pH 7.4)

1 g

1 g

0.5 ミリリットル

0.5 ml

[0030]

As it soaks in solution (second perfusion solution) of below-mentioned composition which at once, designates liver which avulsion is done as temperature -5 deg C, it filled second perfusion solution to liver portal vein with flow of each minute 0.9 ml, 4 min perfusion did.

ジメチルスルホキシド			10 g			
dimethyl sulfoxide			10 g			

D - マンニット D- mannitol			5 g 5 g			
D - グルコース D- glucose			2 g 2 g			
B S A BSA			4.5 g 4.5 g			
酵素阻害剤Pefabloc(Merck) enzyme inhibitor Pefabloc (Merck)			1 mM相当 1 mM equivalent			
p-APMSF p-APMSF			0.1mM相当 0.1 mM equivalent			
Krebs-Henseleit 緩衝液 Krebs-Henseleit buffer			100 ミリリットル 100 ml	(pH (pH		4) 4)

[0031]

(2) low temperature storage

Immediately after perfusion, it stuck liver to metal needle assembly (thickness 5 mm of height 20 mm, metal pedestal of diameter 0.8 mm, center interval 3 mm, needle of gold plating brass needle, needle) and passed.

synthetic paper is stuck beforehand in base of metal needle.

It enclosed this into cold-proof polyethylene bag, in program control freezer which is set to each minute -1 deg C it cooled to -80 deg C.

Pouring solution nitrogen of suitable amount with 3 hours or more passage, later and in cold chest furthermore it cooled.

While adding solution nitrogen, 3 week it preserved liver sample.

[0032]

thawing of (3) tissue

It removed container of sample from in solution nitrogen, after placing in freezer of - 80 deg C for a while, in thawing solution of same composition as first perfusion solution, solution by circulating in case of tissue weight 50 gram with the short time under 2 min temperature of sample entirety it rose to - 4 deg C.

[0033]

In thawing solution it pulled out sample from metal needle assembly, from the indwelling needle to portal vein filled first perfusion solution of temperature -4 deg C with the flow of each minute 1 ml, 4 min perfusion did, substituted second perfusion solution in liver with first perfusion solution.

[0034]

Portion of tissue fixing and stain was done with conventional method, when optical microscope observation it does, immediately after excising by comparison with tissue of (With first perfusion solution only perfusion), change of tissue was not for most part seen.

[0035]

		[実施例 3] [Working Example 3]
(1) (1)	潅	潅流の準備 Preparation of perfusion

[実施例 3]

[Working Example 3]

潅流の準備

Preparation of perfusion

injection doing promazine 1% aqueous solution, blocking peripheral nerve of abdomen, Halothane narcotic doing weight 50 microliter per

100 g injection after doing heparin solution of per ml 1000 IU, with conventional method, it did open abdominal surgery, polyethylene capillary and it inserted indwelling needle which the hitch is done in liver portal vein (portal vein cannulation).

It administered cannulation to also lower aorta with same method and (Lower aorta cannulation),collecting lower aorta and inferior vena cava, ligature it did.

aorta was stopped with forceps, chest was opened and aorta and vena cava was cut off.

[0036]

(2) perfusion

It cut off first perfusion solution of below-mentioned composition which - designates temperature as 5 deg C, via lower aorta cannulation ligating part of inferior vena cava, to liver portal vein, respectively with flow of each minute 1.0ml via portal vein cannulation with flow of each minute 1.5ml in lower aorta, it filled 10 min perfusion did.

From liver blood satisfactory after verifying that it is removed, avulsion did liver from rat.

At time of this portal vein and lower aorta are cut off, guaranteeing each cannulation on liver side.

第一潅流液：
first perfusion solution :
D - グルコース
D- glucose
B S A
BSA
Krebs-Henseleit
Krebs-Henseleit
クロルプロマジン

chlopromazine
2 g
3 g
3 g
100 ミリリットル
100 ml
0.01 g
0.01 g

[0037]

As it soaks in solution (second perfusion solution) of below-mentioned composition which at once, designates liver which avulsion is done as temperature -5 deg C, via lower aorta cannulation with flow of each minute 1.5 ml in lower aorta, it filled this second perfusion solution to liver portal vein, respectively with flow of the each minute 1.0 ml via portal vein cannulation 10 min perfusion did.

ジメチルスルホキシド

dimethyl sulfoxide
D - マンニット
D- mannitol
B S A
BSA
Krebs-Henseleit 緩衝液
Krebs-Henseleit buffer solution
g

g
g
g
g
リリ
Liter

[0038]

(2) low temperature storage

Immediately after perfusion, it stuck liver to metal needle assembly (thickness 5 mm of height 20 mm, metal pedestal of diameter 0.8 mm, center interval 3 mm, needle of gold plating brass needle, needle) and passed.

synthetic paper is stuck beforehand in base of metal needle.

It enclosed this into cold-proof polyethylene bag, in program control freezer which is set to each minute -1 deg C it cooled to - 80 deg C.

Pouring solution nitrogen of suitable amount with 3 hours or more passage, later and in cold chest furthermore it cooled.

While adding solution nitrogen, 3 week it preserved liver sample.

[0039]

thawing of (3) tissue

It removed canister of sample from in solution nitrogen, after placing in freezer of - 80 deg C for a while, it soaked tissue

sample in thawing solution (temperature approximately 30 deg C) of same composition, as first perfusion solution by circulating in case of tissue weight 50 gram with short time under 2 min temperature of sample entirety it rose to 4 deg C.

[0033]

With flow of each minute 1.5ml in lower aorta, it filled to the liver portal vein, respectively with flow of each minute 1.0ml via portal vein cannulation in thawing solution it pulled out sample from metal needle assembly, third perfusion solution of the below-mentioned composition, via lower aorta cannulation 10 min perfusion did, substituted second perfusion solution in liver with third perfusion solution.

temperature of third perfusion solution first made 4 deg C, in perfusion at ratio of per minute approximately 2.5 deg C temperature rose to 25 deg C.

第三潅流液：
third perfusion solution :
D - マンニット
D- mannitol
D - グルコース
D- glucose
ラクトビオン酸
lactobionic acid
B S A
BSA
アスコルビン酸
ascorbic acid
Krebs-Henseleit

Krebs-Henseleit 1 g
1 g
1 g
50 mM
50 mM
3 g
3 g
0.1 g
0.1 g
100 ミリリッ
100 ml

[0040]

Portion of tissue fixing and stain was done with conventional method, when optical microscope observation it does, immediately after excising by comparison with tissue of (With first perfusion solution only perfusion), change of tissue was not for most part seen.

[0044]

[Effects of the Invention]

As for organ, muscle or other organism tissue which is preserved with this invention, characteristic and function of cell are well maintained.

method of this invention effective especially is liver and the preservation of tissue of kidney.

PATENT ABSTRACTS OF JAPAN

(11)Publication number : **08-034701**

(43)Date of publication of application : **06.02.1996**

(21)Application number : **06-323651** (71)Applicant : **SEITAI KAGAKU KENKYUSHO:KK**

(22)Date of filing : **01.12.1994** (72)Inventor : **SHIGEMATSU AKIYO**
SUZUKI SATOSHI
KABURAGI TAKAYUKI
SUEYOSHI TORU

(30)Priority

Priority number :	Priority date :	Priority country :
06 23296	**25.01.1994**	**JP**
06125741	**16.05.1994**	**JP**

(54) STORING OF TISSUE OF ORGANISM

(57)Abstract:
PURPOSE: To provide a method for storing the tissue composing the organ of an organism so as to maintain the characteristics and functions of the tissue at a cell level.
CONSTITUTION: This method for storing the tissue of the organism comprises exchanging blood in the organ of an organism with the first carbohydrate solution not causing the hemolysis of the blood, further exchanging the first carbohydrate solution with the second carbohydrate solution containing an organic solvent to prevent the generation of ice and solute crystals in the tissue, cooling the treated tissue to the temperature of liquid nitrogen, and subsequently storing the frozen tissue. The tissue specimen is thrust with the aggregation of metal needles to uniformly cool the tissue specimen or uniformly and rapidly thaw the frozen tissue specimen.

[JP,08-034701,A]

http://www19.ipdl.ncipi.go.jp/PA1/cgi-bin/PA1DETAIL

[Claim(s)]
[Claim 1] Inject perfusate into the blood vessel which passes to the organ of an animal, and the blood in said organ is permuted by said perfusate. In the living thing organization store method which said a part of organ [at least] excised from said animal is cooled, and temperature is reduced to liquid nitrogen temperature, and saves the organization of said organ The first sugar liquid which does not produce hemolysis substantially as the first perfusate is injected into said blood vessel. In the blood vessel which permutes the blood in the blood vessel in said organ with this first sugar liquid, and passes subsequently to said organ, as the second perfusate The sugar which does not produce a crystal substantially [a water solution does not carry out phase separation in liquid nitrogen temperature, and], The living thing organization store method which mixed liquor with water does not carry out phase separation in liquid nitrogen temperature, and pours in the second sugar liquid containing the organic solvent which does not produce a crystal substantially, permutes said first sugar liquid in said organ with said second sugar liquid, and is characterized by cooling to liquid nitrogen temperature after that.
[Claim 2] The living thing organization store method of claim 1 in which said second sugar liquid contains mannite as said sugar.
[Claim 3] The living thing organization store method of claim 1 in which said second sugar liquid contains an inulin as said sugar.
[Claim 4] The living thing organization store method of claims 1, 2, or 3 said whose organic solvent is dimethyl sulfoxide or a glycerol.
[Claim 5] claim 1 whose temperature of said second sugar liquid is 0 degree C or less thru/or 4 -- one of living thing organization store methods.
[Claim 6] claim 1 which excises all or the predetermined part of said organs from said animal after said blood permutation with said first sugar liquid thru/or 5 -- one of living thing organization store methods.
[Claim 7] claim 1 in which said first sugar liquid contains a glucose thru/or 6 -- one of living thing organization store methods.
[Claim 8] claim 1 in which said first sugar liquid contains the matter which has monoamine oxidase inhibitory action thru/or 7 -- one of living thing organization store methods.
[Claim 9] claim 1 said whose animal is mammalian thru/or 8 -- one of living thing organization store methods.
[Claim 10] Inject perfusate into the blood vessel which passes to the organ of an animal, and the blood in said organ is permuted by said perfusate. In the living thing organization store method which said a part of organ [at least] excised from said animal is cooled, and temperature is reduced to liquid nitrogen temperature, and saves the organization of said organ Intercept the peripheral nerve of the field containing said organ, and the first isotonicity sugar liquid which does not produce hemolysis substantially as the first perfusate in said blood vessel is poured in. In the blood vessel which permutes the blood in the blood vessel in said organ with this first sugar liquid, and passes subsequently to said organ, as the second perfusate The sugar which does not produce a crystal substantially [a water solution does not carry out phase separation in liquid nitrogen

temperature, and], The living thing organization store method which mixed liquor with water does not carry out phase separation in liquid nitrogen temperature, and pours in the second sugar liquid containing the organic solvent which does not produce a crystal substantially, permutes said first sugar liquid in said organ with said second sugar liquid, and is characterized by cooling to liquid nitrogen temperature after that.
[Claim 11] The living thing organization store method of claim 10 in which said second sugar liquid contains mannite as said sugar.
[Claim 12] The living thing organization store method of claims 10 or 11 said whose organic solvent is dimethyl sulfoxide or a glycerol.
[Claim 13] claim 10 whose temperature of said second sugar liquid is 0 degree C or less thru/or 12 -- one of living thing organization store methods.
[Claim 14] claim 10 which excises all or the predetermined part of said organs from said animal after said blood permutation with said first sugar liquid thru/or 13 -- one of living thing organization store methods.
[Claim 15] claim 10 in which said first sugar liquid contains a glucose thru/or 14 -- one of living thing organization store methods.
[Claim 16] claim 10 which intercepts a peripheral nerve by introducing a peripheral nerve cutoff agent into the inside of the blood vessel of said animal, or intraperitoneal thru/or 15 -- one of living thing organization store methods.
[Claim 17] claim 10 which is the matter with which said peripheral nerve cutoff agent has monoamine oxidase inhibitory action thru/or 16 -- one of living thing organization store methods.
[Claim 18] claim 10 said whose animal is mammalian thru/or 17 -- one of living thing organization store methods.
[Claim 19] In the living thing organization store method which a part of organ [at least] excised from the animal is cooled, and temperature is reduced to liquid nitrogen temperature, and is saved The needle aggregate which consists of the metal needle of a large number fixed in parallel with a metal plinth as an organization sample is run through with said a part of excised organ [at least]. The living thing organization store method which said sample with which this needle aggregate was run through is gradually cooled at the rate of predetermined, and temperature is further reduced to liquid nitrogen temperature, and is characterized by saving at liquid nitrogen temperature.
[Claim 20] Said predetermined rate is a living thing organization store method of claim 19 which is the average of less than 1.3 degrees C/m to -80 degrees C.
[Claim 21] In the living thing organization store method which a part of organ [at least] excised from the animal is cooled, and temperature is reduced to liquid nitrogen temperature, and is saved The needle aggregate which consists of the metal needle of a large number fixed in parallel with a metal plinth as an organization sample is run through with said a part of excised organ [at least]. In case cool gradually said sample with which this needle aggregate was run through at the rate of predetermined, temperature is further reduced to liquid nitrogen temperature, it saves at liquid nitrogen temperature and preservation is ended, in order to revive the bioactive of said organization sample, The living thing organization store method characterized by making temperature of the plinth of said needle aggregate into 20 degrees C or more into the time amount which does not exceed 4 minutes.
[Claim 22] The living thing organization store method of claim 21 which makes

temperature of said plinth 20 degrees C or more within 3 minutes in case preservation is ended.

[Claim 23] In the living thing organization store method which perfusate is injected into the blood vessel which passes to the organ of an animal, the blood in said organ is permuted by said perfusate, said a part of organ [at least] excised from said animal is cooled, and temperature is reduced to liquid nitrogen temperature, and is saved The first sugar liquid which does not produce hemolysis substantially as the first perfusate is injected into said blood vessel. In the blood vessel which permutes the blood in the blood vessel in said organ with this first sugar liquid, and passes subsequently to said organ, as the second perfusate The sugar which does not produce a crystal substantially [a water solution does not carry out phase separation in liquid nitrogen temperature, and], Mixed liquor with water does not carry out phase separation in liquid nitrogen temperature, and the second sugar liquid containing the organic solvent which does not produce a crystal substantially is poured in. Said second sugar liquid permutes said first sugar liquid in said organ, and said a part of organ [at least] excised from said animal is made into an organization sample. The living thing organization store method which the organization sample with which ran through with the needle aggregate which consists of the metal needle of a large number fixed in parallel with a metal plinth, and this needle aggregate was run through is gradually cooled at the rate of predetermined, and temperature is further reduced to liquid nitrogen temperature, and is characterized by saving at liquid nitrogen temperature.

[Claim 24] The living thing organization store method of claim 23 said whose organic solvent is dimethyl sulfoxide or a glycerol.

[Claim 25] The living thing organization store method of claims 23 or 24 in which said second sugar liquid contains mannite as said sugar.

[Claim 26] The living thing organization store method of claims 23 or 24 in which said second sugar liquid contains an inulin as said sugar.

[Claim 27] claim 23 in which said first sugar liquid contains mannite as main sugar thru/or 26 -- one of living thing organization store methods.

[Claim 28] claim 23 in which said first sugar liquid contains a glucose thru/or 26 -- one of living thing organization store methods.

[Claim 29] claim 23 said whose predetermined rate is the average of less than 1.3 degrees C/m to -80 degrees C thru/or 28 -- one of living thing organization store methods.

[Claim 30] claim 23 which excises all or the predetermined part of said organs from said animal after said blood permutation with said first sugar liquid thru/or 29 -- one of living thing organization store methods.

[Claim 31] The first sugar liquid which does not produce hemolysis substantially is injected into the blood vessel which passes to the organ of an animal. The sugar which permutes the blood in said organ with said first sugar liquid, excises all or the predetermined part of said organs between this permutation or after a permutation, and subsequently does not produce a crystal substantially [a water solution does not carry out phase separation to said blood vessel in liquid nitrogen temperature, and], Mixed liquor with water does not carry out phase separation in liquid nitrogen temperature, and the second sugar liquid containing the organic solvent which does not produce a crystal substantially is poured in. Said a part of organ [at least] which permuted said first sugar liquid in said organ with said second sugar liquid, and was permuted with this second

sugar liquid as an organization sample The needle aggregate which consists of the metal needle of a large number fixed in parallel with a metal plinth is run through. Said sample with which said needle aggregate was run through is gradually cooled at the rate of predetermined. A part of [at least] temperature of said needle aggregate in the time amount which does not exceed 4 minutes in case temperature is furthermore reduced to liquid nitrogen temperature, it saves at liquid nitrogen temperature and preservation is ended as 20 degrees C or more The living thing organization store method which the temperature of said sample is raised more than the freezing point of said second sugar liquid, and consists of drawing out said sample from said needle aggregate.

[Claim 32] The living thing organization store method of claim 31 said whose organic solvent is dimethyl sulfoxide or a glycerol.

[Claim 33] The living thing organization store method of claims 31 or 32 in which said second sugar liquid contains mannite as said sugar.

[Claim 34] The living thing organization store method of claims 31 or 32 in which said second sugar liquid contains an inulin as said sugar.

[Claim 35] claim 31 whose predetermined rate of said cooling is the average of less than 1.3 degrees C/m to -80 degrees C thru/or 34 -- one of living thing organization store methods.

[Claim 36] claim 31 in which said first sugar liquid contains mannite as main sugar thru/or 35 -- one of living thing organization store methods.

[Claim 37] claim 31 in which said first sugar liquid contains a glucose thru/or 35 -- one of living thing organization store methods.

[Claim 38] claim 31 whose time amount which does not exceed said 4 minutes is less than 3 minutes thru/or 37 -- one of living thing organization store methods.

[Claim 39] The living thing organization store method of claim 38 which makes temperature of the plinth of said needle aggregate 20 degrees C or more within 3 minutes in case preservation is ended.

[Claim 40] The first sugar liquid which does not produce hemolysis substantially is injected into the blood vessel which passes to the organ of an animal. The sugar which permutes the blood in said organ with said first sugar liquid, and subsequently does not produce a crystal substantially [a water solution does not carry out phase separation to said blood vessel in liquid nitrogen temperature, and], Mixed liquor with water does not carry out phase separation in liquid nitrogen temperature, and the second sugar liquid containing the organic solvent which does not produce a crystal substantially is poured in. Excise all or the predetermined part of said organs which permuted said first sugar liquid in said organ with said second sugar liquid, and were permuted with this second sugar liquid from said animal, and this is made into an organization sample. The needle aggregate which consists of the metal needle of a large number fixed in parallel with a metal plinth is run through. Said sample with which said needle aggregate was run through is gradually cooled at the rate of predetermined. In case temperature is furthermore reduced to liquid nitrogen temperature, it saves at liquid nitrogen temperature and preservation is ended, a part of [at least] temperature of said needle aggregate in predetermined time amount as 20 degrees C or more The living thing organization store method which the temperature of said sample is raised more than the freezing point of said second sugar liquid, and consists of drawing out said sample from said needle aggregate.

[Claim 41] The living thing organization store method of claim 40 said whose organic solvent is dimethyl sulfoxide or a glycerol.
[Claim 42] The living thing organization store method of claims 40 or 41 in which said second sugar liquid contains mannite as said sugar.
[Claim 43] The living thing organization store method of claims 40 or 41 which makes temperature of said second sugar liquid 0 degree C or less.
[Claim 44] claim 40 which is that from which said first sugar liquid does not produce a crystal substantially in liquid nitrogen temperature thru/or 43 -- one of living thing organization store methods.
[Claim 45] The living thing organization store method of claim 44 in which said first sugar liquid contains mannite as main sugar.
[Claim 46] The living thing organization store method of claim 44 in which said first sugar liquid contains a glucose.
[Claim 47] The predetermined rate of said cooling is claim 40 thru/or the living thing organization store method of 46 which is the average of less than 1.3 degrees C/m to -80 degrees C.
[Claim 48] claim 40 which makes temperature of the plinth of said needle aggregate 20 degrees C or more within 3 minutes in case preservation is ended thru/or 47 -- one of living thing organization store methods.
[Claim 49] The first sugar liquid which does not produce hemolysis substantially is injected into the blood vessel which passes to the organ of an animal. The sugar which permutes the blood in said organ with said first sugar liquid, and subsequently does not produce a crystal substantially [a water solution does not carry out phase separation to said blood vessel in liquid nitrogen temperature, and], Mixed liquor with water does not carry out phase separation in liquid nitrogen temperature, and the second sugar liquid containing the organic solvent which does not produce a crystal substantially is poured in. All or the predetermined part of said organs which permuted said first sugar liquid in said organ with said second sugar liquid, and were permuted with this second sugar liquid as an organization sample The needle aggregate which consists of the metal needle of a large number fixed in parallel with a metal plinth is run through. Said sample with which said needle aggregate was run through is gradually cooled at the rate of predetermined. The living thing organization store method which temperature is furthermore reduced to liquid nitrogen temperature, and it saves at liquid nitrogen temperature, the temperature of said sample is raised in predetermined time amount more than the freezing point of said second sugar liquid in case preservation is ended, and consists of drawing out said sample from said needle aggregate.
[Claim 50] The living thing organization store method of claim 49 said whose organic solvent is dimethyl sulfoxide or a glycerol.
[Claim 51] The living thing organization store method of claims 49 or 50 in which said second sugar liquid contains mannite as said sugar.
[Claim 52] claim 49 whose predetermined rate of said cooling is the average of less than 1.3 degrees C/m to -80 degrees C thru/or 51 -- one of living thing organization store methods.
[Claim 53] claim 49 in which said first sugar liquid contains a glucose thru/or 52 -- one of living thing organization store methods.
[Claim 54] claim 49 which raises the temperature of said sample within 4 minutes at -5

degrees C or more 5 degrees C or less in case preservation is ended thru/or 53 -- one of living thing organization store methods.
[Claim 55] The living thing organization store method of claim 54 which raises -5 degrees C or more 5 degrees C or less within 4 minutes, and raises the temperature of said sample at 25 degrees C or more at a rate with an average of 4 degrees C [or less]/m in case preservation is ended.
[Claim 56] Inject perfusate into the blood vessel which passes to the organ of an animal, and the blood in said organ is permuted by said perfusate. In the living thing organization store method which said a part of organ [at least] excised from said animal is cooled, and temperature is reduced to liquid nitrogen temperature, and saves some organizations [at least] of said organ Before pouring in said perfusate, intercept the peripheral nerve of the field which contains said organ of said animal beforehand, and the first sugar liquid which does not produce hemolysis substantially is injected into the blood vessel which passes to said organ. The sugar which permutes the blood in said organ with said first sugar liquid, and does not produce a crystal substantially [excise said organ from said animal after that, and a water solution does not carry out phase separation under this permutation or to the blood vessel which passes subsequently to said organ in liquid nitrogen temperature, and], Mixed liquor with water does not carry out phase separation in liquid nitrogen temperature, and the second sugar liquid containing the organic solvent which does not produce a crystal substantially is poured in. Said a part of organ [at least] which permuted said first sugar liquid in said organ with said second sugar liquid, and was permuted with this second sugar liquid as an organization sample The needle aggregate which consists of the metal needle of a large number fixed in parallel with a metal plinth is run through. Said sample with which said needle aggregate was run through is gradually cooled at the rate of predetermined. The living thing organization store method which temperature is furthermore reduced to liquid nitrogen temperature, and it saves at liquid nitrogen temperature, the temperature of said sample is raised in the time amount which does not exceed 4 minutes more than the freezing point of said second sugar liquid in case preservation is ended, and consists of drawing out said sample from said needle aggregate.
[Claim 57] The living thing organization store method of claim 56 said whose organic solvent is dimethyl sulfoxide or a glycerol.
[Claim 58] The living thing organization store method of claims 56 or 57 in which said second sugar liquid contains mannite as said sugar.
[Claim 59] claim 56 in which said first sugar liquid contains a glucose thru/or 58 -- one of living thing organization store methods.
[Claim 60] claim 56 whose predetermined rate of said cooling is the average of less than 1.3 degrees C/m to -80 degrees C thru/or 59 -- one of living thing organization store methods.
[Claim 61] claim 56 which raises the temperature of said sample within 4 minutes at -5 degrees C or more 5 degrees C or less in case preservation is ended thru/or 60 -- one of living thing organization store methods.
[Claim 62] The living thing organization store method of claim 61 which raises -5 degrees C or more 5 degrees C or less within 4 minutes, and raises the temperature of said sample at 25 degrees C or more at a rate with an average of 4 degrees C [or less]/m in case preservation is ended.

[Claim 63] In the living thing organization preservation playback approach of cooling a part of organ [at least] excised from the animal, thawing said organization sample after reducing temperature and saving it to liquid nitrogen temperature, and recovering bioactive Run through the needle aggregate which consists of two or more metal needles fixed in parallel with a metal plinth with all or the predetermined part of said excised organs, and it cools. In case it saves as an organization sample at liquid nitrogen temperature and preservation is ended, in order to revive the bioactive of said organization sample, Said organization sample is thawed using [warm an end, even if there are few said metal needles, and] temperature of said organization sample as -5 degrees C or more 5 degrees C or less within 4 minutes. The living thing organization preservation playback approach which said organization sample is sampled from said needle aggregate, and the temperature of the account organization sample of defrosting back to front is raised at 25 degrees C or more at a rate with an average of 4 degrees C [or less]/m, and is characterized by recovering the bioactive of said organization sample.
[Claim 64] Inject perfusate into the blood vessel which passes to the organ of an animal, and the blood in said organ is permuted by said perfusate. In the living thing organization preservation playback approach of thawing said organization sample and recovering bioactive after cooling said a part of organ [at least] excised from said animal, reducing temperature to liquid nitrogen temperature and saving the organization of said organ Run through the needle aggregate which consists of two or more metal needles fixed in parallel with a metal plinth with all or the predetermined part of said excised organs, and it cools. In case it saves as an organization sample at liquid nitrogen temperature and preservation is ended, an end is warmed even if there are few said metal needles. Said organization sample is thawed using temperature of said organization sample as -5 degrees C or more 5 degrees C or less within 4 minutes. The living thing organization preservation playback approach which the temperature of said organization sample is raised at 25 degrees C or more after it samples said organization sample and 5 minutes pass at least after defrosting since said needle aggregate, and is characterized by recovering the bioactive of said organization sample.
[Claim 65] The living thing organization preservation playback approach of claim 64 of recovering the bioactive of said organization sample by carrying out perfusion of the buffer solution which contains sugar and a serum protein in the blood vessel which passes to said organization sample while raising the temperature after defrosting to 25 degrees C.
[Claim 66] The living thing organization preservation playback approach of claim 65 that said buffer solution contains dibutyryl cyclic AMP.

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to the cold storage approach and the preservation playback approach of the living thing organization which can maintain the property and function of a cell which constitute a living thing organization store method, especially an organization.
[0002]
[Description of the Prior Art] Since cells, such as microorganisms, such as bacteria, and

an ovum, a sperm, a cultured cell, are saved so that activity may be maintained 50% or more, a cell is gradually cooled with the speed around 1 degree C/m, it saves around -200 degrees C, and the approach of dissolving quickly (defrosting) is learned at the time of preservation termination. Under the present circumstances, in order to prevent destruction by freezing of a cell, a cell is placed into 10% glycerol or 5 - 10% dimethyl sulfoxide water solution.

[0003]

[Problem(s) to be Solved by the Invention] The cell according to individuals, such as a microorganism, an ovum, and a sperm, can be comparatively saved by the above-mentioned approach for a long period of time. However, when such liquid was injected into organs, such as the organ of an animal, for example, liver, and the kidney, the property of the cell of an organization and the loss of a function which were saved were remarkable.

[0004] The purpose of this invention is offering the approach of saving the organization which constitutes the organ of a living thing so that the property's and function's may be maintained on cell level. Moreover, the purpose of this invention is offering the approach of recovering bioactive, after saving the organization which constitutes the organ of a living thing so that the property and function may be maintained on cell level.

[0005]

[Means for Solving the Problem] In order that the living thing organization store method of this invention may attain this purpose, the first sugar liquid which does not produce hemolysis substantially is injected into the blood vessel which passes to the predetermined organ of an animal. The sugar which permutes the blood in an organ with the first sugar liquid, and does not produce a crystal substantially [a water solution does not carry out phase separation in liquid nitrogen temperature, and], Mixed liquor with water does not carry out phase separation in liquid nitrogen temperature, and the second sugar liquid containing the organic solvent which does not produce a crystal substantially is injected into a blood vessel. The second sugar liquid permutes the first sugar liquid in a blood vessel, and all or the predetermined part of organs is excised. The needle aggregate which consists of the metal needle of a large number fixed in parallel is run through with the sample of this excised organ. In case cool gradually at the rate of predetermined, temperature is further reduced to liquid nitrogen temperature, the sample with which the needle aggregate was run through is saved at liquid nitrogen temperature and preservation is ended, a part of [at least] temperature of the needle aggregate is quickly made into 20 degrees C or more. The temperature of a sample is promptly raised to more than the freezing point of the second sugar liquid, and it consists of drawing out a sample from the needle aggregate. This invention is applicable not only to organs, such as an organ of an animal, but connective tissue, such as muscles.

[0006] Instead of excising an organization sample after a permutation (henceforth perfusion) with the first [of blood], and second sugar liquid, all or the predetermined part of organs may be excised from an animal between the perfusion before perfusion. A predetermined part is one of the hepatic lobes in liver, or two. In order to perform perfusion or excision, it is necessary to conduct an operation for an animal. When performing perfusion after excision, some organizations may be further excised as a sample after perfusion.

[0007] Before undergoing an operation, it is desirable to intercept the peripheral nerve

near [used as especially an object] the organ of an animal because of maintenance of an organization function. Cutoff of the peripheral nerve near an object organ can be attained by pouring into the inside of a blood vessel, or intraperitoneal the matter which has monoamine oxidase (following, MAO, and brief sketch) inhibitory action. There are for example, N-amino alkyl phenothiazin system compound, N-amino alkyl JIBENZOAZA cycloheptadiene system compound, an isonicotinic acid hydrazide system compound, and a propargyl amine derivative in MAO inhibitor applicable to this invention. For example, they are chlorpromazine, a promethazine, imipramine, the iproniazid, pargyline, etc. MAO inhibitor may be added also in perfusate.

[0008] Perfusion usually stabs with a detention needle (hollow) the blood vessel which passes to an organ, and is performed. A flow rate is chosen according to the class of organ, the class of blood vessel, and a size. The permutation with the first and second sugar liquid is enough performed so that blood and the first sugar liquid may not remain substantially, respectively.

[0009] The sugar contained in the second sugar liquid must be sugar which does not produce a crystal substantially [in liquid nitrogen temperature, do not carry out phase separation, and] like mannite (mannitol) or an inulin. If a crystal is not produced substantially, it means not producing the crystal of extent which does damage to the structure and bioactive of an organization after preservation. As for this sugar, what does not need to permeate intracellular and cannot permeate intracellular easily rather is desirable.

[0010] As for the first sugar liquid, it is desirable that sugar which is useful to maintenance of the bioactive of an organization, for example, a glucose, is included. As for the concentration of a glucose, it is desirable to consider as the nearest possible concentration equally to the glucose concentration under organization. The first sugar liquid needs to consider as the presentation which does not cause hemolysis constituting the cause of a residual of the corpuscle component of a under [an organization]. If hemolysis is not produced substantially, it means that there are not structure of the organization by hemolysis and damage on bioactive.

[0011] Although it is desirable that it is an isotonicity (isotonic) to an organization substantially as for the first and second sugar liquid, what is necessary is just 1/2 of blood or lymph thru/or the osmotic pressure of the twice as many range as this. The first and second sugar liquid may also contain salts besides sugar, an acid, a base, a buffer, a surfactant, a thickener, etc., respectively. As for the first [which is used for perfusion], and second temperature of sugar liquid, it is desirable to maintain at as low the temperature more than the freezing point of liquid (usually -10 degrees C thru/or +5 degrees C) as possible in order to prevent change of an organization.

[0012] Mixed liquor with water must not carry out phase separation of the organic solvent used for the second sugar liquid in liquid nitrogen temperature, and it must not produce a crystal substantially. If a crystal is not produced substantially, it means not producing the crystal of extent which does damage to the structure and bioactive of an organization after preservation. Organic solvents equipped with such a property are a glycerol and dimethyl sulfoxide. Although 5 - 10% of the concentration of the organic solvent in the second sugar liquid is suitable, ** is also highly (for example, 15%) good a little lower (for example, 3%) a little than this.

[0013] As for the metal needle and metal plinth which are used for the needle aggregate,

what consists of the metal from which a front face does not affect an organization and honeydew, and produces neither rust nor corrosion by them at least is desirable. For example, stainless steel, gold, platinum, gilding, chrome plating (iron, brass, etc.), etc. are used.

[0014] The size of a metal needle needs to be taken as the size which gives sufficient mechanical strength to run through with an organization sample. Heat conduction is so prompt that spacing is so narrow that a metal needle is thick, and although the temperature of an organization sample can be quickly raised in case preservation at low temperature is ended, the mechanical damage of the whole organization sample becomes large. The size of a metal needle is 0.2. There is nothing and 1.6 mm and spacing (center to center) are 0.5. Or 4mm is suitable.

[0015] As for cooling of the organization sample with which the metal needle was run through, it is desirable to perform even -80 degrees C gradually at least, for example, it is desirable to consider a temperature fall as the average of less than 1.3 degrees C/m to -80 degrees C. Such a temperature fall stores a sample in the heat insulation box of suitable magnitude, and if it places all over a -80-degree C freezer, it is realizable. The freezer by which program control was carried out may be used.

[0016] In case preservation is ended, by making quickly a part of [at least] temperature of the needle aggregate into 20 degrees C or more within for example, 2 minutes, to the temperature more than the melting point of the second sugar liquid (usually - 5 degrees C thru/or +5 degrees C, for example, 4 degrees C), the temperature of a sample is raised quickly and thawed. Specifically, a suitable quantity of tepid water (20 degrees C thru/or 40 degrees C) (liquids other than water are sufficient) is poured around a metal plinth. Or the perfusate (sugar liquid) of the suitable temperature for the surroundings of the sample run through by the metal needle aggregate is passed. By such approach, the temperature of an organization sample is preferably raised within 4 minutes more than the freezing point of the second sugar liquid, and is thawed.

[0017] After a sample dissolves, an organization sample is drawn out from the needle aggregate by drawing out the cloth and synthetic paper with which are a suitable means, for example, the base of a metal needle was run through. Perfusion of the drawn-out organization sample is carried out by suitable temperature, for example, a 4-degree C physiological saline, sugar liquid, tissue culture liquid, and others, it recovers the bioactive needed at least and the business after it is presented with it.

[0018] Carrying out perfusion with the liquid (for example, the same liquid as the first sugar liquid) of a suitable presentation, in order to recover bioactive, temperature is raised over the time amount more than the minimum, and it is made to go up to near 37 more degree C from temperature required for defrosting of an organization to about 25 degrees C. The minimum time amount is 5 minutes and it is desirable to raise temperature to 25 degrees C over 10 minutes or more. There is no limit of time amount especially in the rise to near 37 degree C from the temperature of 25 degrees C. dibutyryl cyclicAMP may also be included in the perfusate used in this phase, and it is useful to prevention of the cell membrane damage under hypoxia concentration. hydrocortizone may also be included in perfusate again.

[0019] Especially this invention is useful to cold storage, such as the liver of mammalian, the kidney, the pancreas, a spleen, a testis, the ovary, a suprarenal gland, a brain, and the thyroid. It is effective for especially the cold storage of liver.

[0020]
[Function] The first sugar liquid is injected into the blood vessel which passes to the organ of an animal, and the blood in an organ is eliminated by permuting blood with the first sugar liquid. By injecting the second sugar liquid into a blood vessel furthermore, and permuting the first sugar liquid with the second sugar liquid, the first sugar liquid is eliminated and the inside of a blood vessel is filled with the second sugar liquid. A sinusoid and an intercellular space are also filled with the second sugar liquid at this time.
[0021] If the needle aggregate which consists of the metal needle of a large number fixed in parallel with a metal plinth is run through with the organization sample excised (a perfusion front stirrup may be excised in perfusion) and it cools gradually, the temperature of the whole organization sample will be comparatively maintained at homogeneity by heat conduction through the run-through metal needle. The organization sample run through by the needle aggregate is saved at liquid nitrogen temperature by reducing temperature further to liquid nitrogen temperature, and maintaining at this temperature.
[0022] Since the second sugar liquid introduced into the organization from the blood vessel contains the sugar which does not produce a crystal substantially [a water solution does not carry out phase separation in liquid nitrogen temperature, and], the crystal of sugar itself and ice does not produce it at liquid nitrogen temperature at least besides the cell of an organization (a sinusoid and an intercellular space are included in a blood vessel). Moreover, it mixes with the second sugar liquid with water well, and mixed liquor with water does not carry out phase separation in liquid nitrogen temperature, and it contains the organic solvent which does not produce a crystal substantially. This organic solvent permeates intracellular. Since this organic solvent exists, the inside and outside of a cell do not produce an ice or solvent (or eutectic object) crystal during an organization in liquid nitrogen temperature, either. So, destruction of the cell by generation of ice and a solvent crystal is prevented.
[0023] If a part of [at least] temperature of the needle aggregate is quickly made into 20 degrees C or more in case preservation is ended, the temperature of an organization sample will rise to more than the melting point of the second sugar liquid, and if the part which touches a metal needle dissolves, a sample can be drawn out from the needle aggregate. If the temperature of an organization is furthermore raised at 25 degrees C or more, an organization will recover the bioactive. By heat conduction through the run-through metal needle aggregate, the temperature of the whole organization sample rises to homogeneity promptly and comparatively compared with the case where the metal needle aggregate is not used. For this rapid and uniform temperature rise of the whole sample, the property and function of a tissue cell are not spoiled and adjustment of subsequent environments (temperature etc.) can recover them.
[0024]
[Example] An example is shown below and it considers as still more concrete explanation of this invention.
[Example 1]
(1) a with a manufacture outer-diameter die length [of the metal needle aggregate / 15mm die length of 0.3mm] stainless steel hypodermic needle (what excluded the connection section to a glass syringe) -- the plinth of low melting alloys -- 1mm spacing -- length -- width 12 train erection was carried out ten train (a tip -- facing up). In order to

take out the inserted organization sample, the glass rod of 0.6mm of sizes was inserted in the shape of a grid between needles along with the plinth.
[0025] (2) liver perfusion -- the laparotomy was performed on the healthy rat and the detention needle was inserted in the hepatic portal vein. According to the conventional method of liver perfusion, by the flow rate of 4ml/m, the first perfusate of the following presentation made into the temperature of 4 degrees C was poured into the hepatic portal vein, and carried out perfusion for 5 minutes from the polyethylene capillary connected with the detention needle.
D-mannitol 1 gKrebs-Ringer buffer solution 20ml, subsequently, by the flow rate of 4ml/m, the second perfusate of the following presentation made into the temperature of 4 degrees C was poured into the hepatic portal vein, and carried out perfusion for 5 minutes.
Dimethyl sulfoxide 2gD-mannite 1 gKrebs-Ringer buffer solution 20ml [0026] (3) Liver was extracted immediately after cold storage perfusion, and the metal needle aggregate was run through. After storing this in the form polystyrene heat insulation box, it stored all over the -80-degree C freezer (slowly cooled at less than 1 degree C/m). The heat insulation box was filled with the liquid nitrogen of optimum dose after 3-hour or more progress, and it cooled further. The liver sample was saved for three weeks, filling up liquid nitrogen.
[0027] (4) The wave plate was fixed to the location with a depth [of a constant temperature bath with a defrosting temperature / of an organization / of 25 degrees C] of 2 millimeters. The liver sample which it let pass to the metal needle was taken out from the heat insulation box as it was, and it carried on this wave plate. the 25-degree C constant temperature to which a metal plinth flows around -- it is heated with water. It took out after 2 minutes, the liver sample was extruded using the grid of the glass rod inserted in the plinth side of a metal needle, and it removed from the needle. Perfusion of the first perfusate with a temperature of 4 degrees C was poured in and carried out to liver for 5 minutes by the flow rate of 4ml/m from the portal vein the bottom picking outside, and the second perfusate in liver was permuted by the first perfusate.
[0028] (5) After carrying out formalin fixation of the check liver sample of organization preservation, it dyed by making it an intercept with a thickness of 5 microns. As a result of microscope observation, the cell of liver is saved well and especially the glycogen granule was accepted clearly.
[0029] [Example 2]
(1) organization extraction and perfusion -- the abdominal cavity of a healthy rat was injected with 0.1ml of chloro bromazine 1% water solutions, and the weight of 100g, the abdominal peripheral nerve was intercepted, after injecting with and anesthetizing 0.04mg [per weight of 100g] SOMUNO pentyl, the laparotomy was performed and the detention needle connected with the polyethylene capillary was inserted in the hepatic portal vein. According to the conventional method of liver perfusion, it is per minute about the first perfusate of the following presentation which made temperature -5 degrees C from the polyethylene capillary connected with the detention needle. By the flow rate of 0.9ml, it poured into the hepatic portal vein and perfusion was carried out for 4 minutes. while carrying out perfusion -- liver -- the law from a rat -- it excised by the method.
D-glucose 2 gBSA 4.5 g enzyme inhibitor Pefabloc (Merck) 1 mM equivalent p-APMSF

0.1 mM equivalent (p-amidinophenylmethanesulfonylfluoride hydrochloride) Krebs-Henseleit Buffer solution 100 Milliliter (pH 7.4) Chloro bromazine 1 g heparin 0.5 Milliliter [0030] While being immersed into the liquid (the second perfusate) of the following presentation which made extracted liver immediately the temperature of -5 degrees C, it is per minute about the second perfusate. By the flow rate of 0.9ml, it poured into the hepatic portal vein and perfusion was carried out for 4 minutes. Dimethyl sulfoxide 10gD-mannite 5gD-glucose 2 gBSA 4.5g enzyme inhibitor Pefabloc (Merck) 1 mM equivalent p-APMSF It is Krebs-Henseleit by 0.1mM. Buffer solution 100 Milliliter (pH 7.4) [0031] (2) The metal needle aggregate (diameter of a gilding brass needle and a needle 5mm in 0.8mm, main spacing of 3mm, height of 20mm of a needle, thickness of a metal plinth) was run through with liver immediately after cold storage perfusion. The base of a metal needle is beforehand stabbed with the synthetic paper. This was enclosed with the cold-resistance polyethylene bag, and it cooled to -80 degrees C all over the program control freezer set as -1 degree C per minute. After 3-hour or more progress, in the heat insulation box, the liquid nitrogen of optimum dose was poured out and it cooled further. The liver sample was saved for three weeks, filling up liquid nitrogen.

[0032] (3) After taking out the container of a sample out of the defrosting liquid nitrogen of an organization and placing for a while all over a -80-degree C freezer, in the case of 50g organization weight, the temperature of the whole sample was raised to -4 degrees C in a short time for less than 2 minutes by circulating liquid in the defrosting liquid of the same presentation as the first perfusate.

[0033] The sample was sampled from the metal needle aggregate in defrosting liquid, perfusion of the first perfusate with a temperature of -4 degrees C was poured in and carried out from the detention needle for 4 minutes by the flow rate of 1ml/m to the portal vein, and the second perfusate in liver was permuted by the first perfusate.

[0034] When some organizations were fixed and dyed with the conventional method and optical microscope observation was carried out, most change of an organization was not seen as compared with the organization immediately after excision (only perfusion by the first perfusate).

[0035] [Example 3]

(1) preparation of perfusion -- the abdominal cavity of a healthy rat was injected with the 0.1ml [per weight of 100g] chlorpromazine 1% water solution, the abdominal peripheral nerve was intercepted, per ml, after carrying out 50 microliter injection per weight of 100g, halothane anesthesia of the heparin liquid of 1000IU was carried out with the conventional method, the laparotomy was performed, and the detention needle connected with the polyethylene capillary was inserted in the hepatic portal vein (portal vein cannulation). Cannulation was performed also to the bottom main artery by the same approach (bottom main artery cannulation), and the bottom main artery and the inferior vena cava were litigated collectively. The main artery was stopped by the crane mel, the thorax was opened and a main artery and vena cava were cut.

[0036] (2) Cut the ligation section of a perfusion inferior vena cava, lead bottom main artery cannulation in the first perfusate of the following presentation which made temperature -5 degrees C, and it is per minute. Portal vein cannulation is led to a bottom main artery by the flow rate of 1.5ml, and it is per minute. By the flow rate of 1.0ml, it poured into the hepatic portal vein, respectively, and perfusion was carried out for 10

minutes. After checking that blood had been enough eliminated from liver, liver was extracted from the rat. At this time, a portal vein and a bottom main artery secure each cannulation to a liver side, and are cut.
The first perfusate: D-glucose 2 gBSA 3 gKrebs-Henseleit Buffer solution 100 Milliliter (pH 7.4) Chlorpromazine 0.01g [0037] While being immersed into the liquid (the second perfusate) of the following presentation which made extracted liver immediately the temperature of -5 degrees C, bottom main artery cannulation is led in this second perfusate, and it is per minute. Portal vein cannulation is led to a bottom main artery by the flow rate of 1.5ml, and it is per minute. By the flow rate of 1.0ml, it poured into the hepatic portal vein, respectively, and perfusion was carried out for 10 minutes.
Dimethyl sulfoxide 10 gD-mannite 5 gBSA 3 gKrebs-Henseleit Buffer solution 100 Milliliter (pH 7.4) [0038] (2) The metal needle aggregate (diameter of a gilding brass needle and a needle 5mm in 0.8mm, main spacing of 3mm, height of 20mm of a needle, thickness of a metal plinth) was run through with liver immediately after cold storage perfusion. The base of a metal needle is beforehand stabbed with the synthetic paper. This was enclosed with the cold-resistance polyethylene bag, and it cooled to -80 degrees C all over the program control freezer set as -1 degree C per minute. After 3-hour or more progress, in the heat insulation box, the liquid nitrogen of optimum dose was poured out and it cooled further. The liver sample was saved for three weeks, filling up liquid nitrogen.
[0039] (3) After taking out the container of a sample out of the defrosting liquid nitrogen of an organization and placing for a while all over a -80-degree C freezer, the organization sample was dipped in the defrosting liquid (temperature of about 30 degrees C) of the same presentation as the first perfusate, and, in the case of 50g organization weight, the temperature of the whole sample was raised to 4 degrees C in a short time for less than 2 minutes by circulating liquid.
[0033] A sample is sampled from the metal needle aggregate in defrosting liquid, bottom main artery cannulation is led in the third perfusate of the following presentation, and it is per minute. Portal vein cannulation is led to a bottom main artery by the flow rate of 1.5ml, and it is per minute. By the flow rate of 1.0ml, it poured into the hepatic portal vein, respectively, perfusion was carried out for 10 minutes, and the second perfusate in liver was permuted by the third perfusate. Temperature of the third perfusate was made into 4 degrees C at first, and raised temperature to 25 degrees C at a rate of about 2.5 degrees C per minute in perfusion.
The third perfusate: D-mannitol 1 gD-glucose 1 g lactobionate 50 mMBSA 3 g ascorbic acid 0.1 gKrebs-Henseleit Buffer solution 100 Milliliter (pH 7.4) [0040] When some organizations were fixed and dyed with the conventional method and optical microscope observation was carried out, most change of an organization was not seen as compared with the organization immediately after excision (only perfusion by the first perfusate).
[0041] They are the temperature of 37 degrees C, and relative humidity about the liver which finished perfusion by the third perfusate in the example 3. It stores into 100% of box, and is [2-14C] of 185kBq. Diazepam was poured in from portal vein cannulation and the perfusate for a metabolic turnover experiment of the following presentation was supplied by the flow rate of 1 ml/min using the immediately after metering pump. This liquid was supplied to coincidence by the flow rate of 1.5 ml/min through artery cannulation. Perfusion was continued for 20 minutes, the perfusate which flows out from

liver at this time was received in ROUTO, and it extracted in the test tube every 2 minutes.
[0042] The extracted perfusate carried out at-long-intervals alignment separation by 3000 rotations for 10 minutes, separated the radioactive metabolite in the supernatant liquid by thin-layer chromatography, and performed detection analysis by radio RUMINOGURAFI. Consequently, 4'-hydroxydiazepam and Nordazepam which are the metabolite besides Diazepam which is a parent compound in effluent, Temazepam, and Oxazepam It was detected. These are metabolite accepted when the same experiment is conducted using the liver which does not carry out cryopreservation, and show that the liver tissue saved by this approach has metabolic activity.
[0043] Moreover, any effluent is abbreviation when the quantum of the lactate dehydrogenase (LDH) was carried out. It is 100IU / liter and normal values were shown through the perfusion for 20 minutes. This shows that liver tissue has not received damage even if it passes through the process of freezing and defrosting.
[0044]
[Effect of the Invention] As for living thing organizations saved by this invention, such as an organ and muscles, the property and function of the cell are often maintained. Especially the approach of this invention is effective in preservation of the tissue of liver and the kidney.

[Industrial Application] This invention relates to the cold storage approach and the preservation playback approach of the living thing organization which can maintain the property and function of a cell which constitute a living thing organization store method, especially an organization.

[Description of the Prior Art] Since cells, such as microorganisms, such as bacteria, and an ovum, a sperm, a cultured cell, are saved so that activity may be maintained 50% or more, a cell is gradually cooled with the speed around 1 degree C/m, it saves around -200 degrees C, and the approach of dissolving quickly (defrosting) is learned at the time of preservation termination. Under the present circumstances, in order to prevent destruction by freezing of a cell, a cell is placed into 10% glycerol or 5 - 10% dimethyl sulfoxide water solution.

[Effect of the Invention] As for living thing organizations saved by this invention, such as an organ and muscles, the property and function of the cell are often maintained. Especially the approach of this invention is effective in preservation of the tissue of liver and the kidney.

[Problem(s) to be Solved by the Invention] The cell according to individuals, such as a microorganism, an ovum, and a sperm, can be comparatively saved by the above-mentioned approach for a long period of time. However, when such liquid was injected into organs, such as the organ of an animal, for example, liver, and the kidney, the property of the cell of an organization and the loss of a function which were saved were remarkable.

[0004] The purpose of this invention is offering the approach of saving the organization which constitutes the organ of a living thing so that the property's and function's may be maintained on cell level. Moreover, the purpose of this invention is offering the approach of recovering bioactive, after saving the organization which constitutes the organ of a living thing so that the property and function may be maintained on cell level.

[Means for Solving the Problem] In order that the living thing organization store method of this invention may attain this purpose, the first sugar liquid which does not produce hemolysis substantially is injected into the blood vessel which passes to the predetermined organ of an animal. The sugar which permutes the blood in an organ with the first sugar liquid, and does not produce a crystal substantially [a water solution does not carry out phase separation in liquid nitrogen temperature, and], Mixed liquor with water does not carry out phase separation in liquid nitrogen temperature, and the second sugar liquid containing the organic solvent which does not produce a crystal substantially is injected into a blood vessel. The second sugar liquid permutes the first sugar liquid in a blood vessel, and all or the predetermined part of organs is excised. The needle aggregate which consists of the metal needle of a large number fixed in parallel is run through with the sample of this excised organ. In case cool gradually at the rate of predetermined, temperature is further reduced to liquid nitrogen temperature, the sample with which the needle aggregate was run through is saved at liquid nitrogen temperature and preservation is ended, a part of [at least] temperature of the needle aggregate is quickly made into 20 degrees C or more. The temperature of a sample is promptly raised to more than the freezing point of the second sugar liquid, and it consists of drawing out a sample from the needle aggregate. This invention is applicable not only to organs, such as an organ of an animal, but connective tissue, such as muscles.

[0006] Instead of excising an organization sample after a permutation (henceforth perfusion) with the first [of blood], and second sugar liquid, all or the predetermined part of organs may be excised from an animal between the perfusion before perfusion. A predetermined part is one of the hepatic lobes in liver, or two. In order to perform perfusion or excision, it is necessary to conduct an operation for an animal. When performing perfusion after excision, some organizations may be further excised as a sample after perfusion.

[0007] Before undergoing an operation, it is desirable to intercept the peripheral nerve near [used as especially an object] the organ of an animal because of maintenance of an organization function. Cutoff of the peripheral nerve near an object organ can be attained by pouring into the inside of a blood vessel, or intraperitoneal the matter which has

monoamine oxidase (following, MAO, and brief sketch) inhibitory action. There are for example, N-amino alkyl phenothiazin system compound, N-amino alkyl JIBENZOAZA cycloheptadiene system compound, an isonicotinic acid hydrazide system compound, and a propargyl amine derivative in MAO inhibitor applicable to this invention. For example, they are chlorpromazine, a promethazine, imipramine, the iproniazid, pargyline, etc. MAO inhibitor may be added also in perfusate.

[0008] Perfusion usually stabs with a detention needle (hollow) the blood vessel which passes to an organ, and is performed. A flow rate is chosen according to the class of organ, the class of blood vessel, and a size. The permutation with the first and second sugar liquid is enough performed so that blood and the first sugar liquid may not remain substantially, respectively.

[0009] The sugar contained in the second sugar liquid must be sugar which does not produce a crystal substantially [in liquid nitrogen temperature, do not carry out phase separation, and] like mannite (mannitol) or an inulin. If a crystal is not produced substantially, it means not producing the crystal of extent which does damage to the structure and bioactive of an organization after preservation. As for this sugar, what does not need to permeate intracellular and cannot permeate intracellular easily rather is desirable.

[0010] As for the first sugar liquid, it is desirable that sugar which is useful to maintenance of the bioactive of an organization, for example, a glucose, is included. As for the concentration of a glucose, it is desirable to consider as the nearest possible concentration equally to the glucose concentration under organization. The first sugar liquid needs to consider as the presentation which does not cause hemolysis constituting the cause of a residual of the corpuscle component of a under [an organization]. If hemolysis is not produced substantially, it means that there are not structure of the organization by hemolysis and damage on bioactive.

[0011] Although it is desirable that it is an isotonicity (isotonic) to an organization substantially as for the first and second sugar liquid, what is necessary is just 1/2 of blood or lymph thru/or the osmotic pressure of the twice as many range as this. The first and second sugar liquid may also contain salts besides sugar, an acid, a base, a buffer, a surfactant, a thickener, etc., respectively. As for the first [which is used for perfusion], and second temperature of sugar liquid, it is desirable to maintain at as low the temperature more than the freezing point of liquid (usually -10 degrees C thru/or +5 degrees C) as possible in order to prevent change of an organization.

[0012] Mixed liquor with water must not carry out phase separation of the organic solvent used for the second sugar liquid in liquid nitrogen temperature, and it must not produce a crystal substantially. If a crystal is not produced substantially, it means not producing the crystal of extent which does damage to the structure and bioactive of an organization after preservation. Organic solvents equipped with such a property are a glycerol and dimethyl sulfoxide. Although 5 - 10% of the concentration of the organic solvent in the second sugar liquid is suitable, ** is also highly (for example, 15%) good a little lower (for example, 3%) a little than this.

[0013] As for the metal needle and metal plinth which are used for the needle aggregate, what consists of the metal from which a front face does not affect an organization and honeydew, and produces neither rust nor corrosion by them at least is desirable. For example, stainless steel, gold, platinum, gilding, chrome plating (iron, brass, etc.), etc. are

used.
[0014] The size of a metal needle needs to be taken as the size which gives sufficient mechanical strength to run through with an organization sample. Heat conduction is so prompt that spacing is so narrow that a metal needle is thick, and although the temperature of an organization sample can be quickly raised in case preservation at low temperature is ended, the mechanical damage of the whole organization sample becomes large. The size of a metal needle is 0.2. There is nothing and 1.6 mm and spacing (center to center) are 0.5. Or 4mm is suitable.
[0015] As for cooling of the organization sample with which the metal needle was run through, it is desirable to perform even -80 degrees C gradually at least, for example, it is desirable to consider a temperature fall as the average of less than 1.3 degrees C/m to -80 degrees C. Such a temperature fall stores a sample in the heat insulation box of suitable magnitude, and if it places all over a -80-degree C freezer, it is realizable. The freezer by which program control was carried out may be used.
[0016] In case preservation is ended, by making quickly a part of [at least] temperature of the needle aggregate into 20 degrees C or more within for example, 2 minutes, to the temperature more than the melting point of the second sugar liquid (usually - 5 degrees C thru/or +5 degrees C, for example, 4 degrees C), the temperature of a sample is raised quickly and thawed. Specifically, a suitable quantity of tepid water (20 degrees C thru/or 40 degrees C) (liquids other than water are sufficient) is poured around a metal plinth. Or the perfusate (sugar liquid) of the suitable temperature for the surroundings of the sample run through by the metal needle aggregate is passed. By such approach, the temperature of an organization sample is preferably raised within 4 minutes more than the freezing point of the second sugar liquid, and is thawed.
[0017] After a sample dissolves, an organization sample is drawn out from the needle aggregate by drawing out the cloth and synthetic paper with which are a suitable means, for example, the base of a metal needle was run through. Perfusion of the drawn-out organization sample is carried out by suitable temperature, for example, a 4-degree C physiological saline, sugar liquid, tissue culture liquid, and others, it recovers the bioactive needed at least and the business after it is presented with it.
[0018] Carrying out perfusion with the liquid (for example, the same liquid as the first sugar liquid) of a suitable presentation, in order to recover bioactive, temperature is raised over the time amount more than the minimum, and it is made to go up to near 37 more degree C from temperature required for defrosting of an organization to about 25 degrees C. The minimum time amount is 5 minutes and it is desirable to raise temperature to 25 degrees C over 10 minutes or more. There is no limit of time amount especially in the rise to near 37 degree C from the temperature of 25 degrees C. dibutyryl cyclicAMP may also be included in the perfusate used in this phase, and it is useful to prevention of the cell membrane damage under hypoxia concentration. hydrocortizone may also be included in perfusate again.
[0019] Especially this invention is useful to cold storage, such as the liver of mammalian, the kidney, the pancreas, a spleen, a testis, the ovary, a suprarenal gland, a brain, and the thyroid. It is effective for especially the cold storage of liver.

[Function] The first sugar liquid is injected into the blood vessel which passes to the organ of an animal, and the blood in an organ is eliminated by permuting blood with the first sugar liquid. By injecting the second sugar liquid into a blood vessel furthermore, and permuting the first sugar liquid with the second sugar liquid, the first sugar liquid is eliminated and the inside of a blood vessel is filled with the second sugar liquid. A sinusoid and an intercellular space are also filled with the second sugar liquid at this time.
[0021] If the needle aggregate which consists of the metal needle of a large number fixed in parallel with a metal plinth is run through with the organization sample excised (a perfusion front stirrup may be excised in perfusion) and it cools gradually, the temperature of the whole organization sample will be comparatively maintained at homogeneity by heat conduction through the run-through metal needle. The organization sample run through by the needle aggregate is saved at liquid nitrogen temperature by reducing temperature further to liquid nitrogen temperature, and maintaining at this temperature.
[0022] Since the second sugar liquid introduced into the organization from the blood vessel contains the sugar which does not produce a crystal substantially [a water solution does not carry out phase separation in liquid nitrogen temperature, and], the crystal of sugar itself and ice does not produce it at liquid nitrogen temperature at least besides the cell of an organization (a sinusoid and an intercellular space are included in a blood vessel). Moreover, it mixes with the second sugar liquid with water well, and mixed liquor with water does not carry out phase separation in liquid nitrogen temperature, and it contains the organic solvent which does not produce a crystal substantially. This organic solvent permeates intracellular. Since this organic solvent exists, the inside and outside of a cell do not produce an ice or solvent (or eutectic object) crystal during an organization in liquid nitrogen temperature, either. So, destruction of the cell by generation of ice and a solvent crystal is prevented.
[0023] If a part of [at least] temperature of the needle aggregate is quickly made into 20 degrees C or more in case preservation is ended, the temperature of an organization sample will rise to more than the melting point of the second sugar liquid, and if the part which touches a metal needle dissolves, a sample can be drawn out from the needle aggregate. If the temperature of an organization is furthermore raised at 25 degrees C or more, an organization will recover the bioactive. By heat conduction through the run-through metal needle aggregate, the temperature of the whole organization sample rises to homogeneity promptly and comparatively compared with the case where the metal needle aggregate is not used. For this rapid and uniform temperature rise of the whole sample, the property and function of a tissue cell are not spoiled and adjustment of subsequent environments (temperature etc.) can recover them.

[Example] An example is shown below and it considers as still more concrete explanation of this invention.
[Example 1]
(1) a with a manufacture outer-diameter die length [of the metal needle aggregate / 15mm die length of 0.3mm] stainless steel hypodermic needle (what excluded the connection section to a glass syringe) -- the plinth of low melting alloys -- 1mm spacing -

- length -- width 12 train erection was carried out ten train (a tip -- facing up). In order to take out the inserted organization sample, the glass rod of 0.6mm of sizes was inserted in the shape of a grid between needles along with the plinth.
[0025] (2) liver perfusion -- the laparotomy was performed on the healthy rat and the detention needle was inserted in the hepatic portal vein. According to the conventional method of liver perfusion, by the flow rate of 4ml/m, the first perfusate of the following presentation made into the temperature of 4 degrees C was poured into the hepatic portal vein, and carried out perfusion for 5 minutes from the polyethylene capillary connected with the detention needle.
D-mannitol 1 gKrebs-Ringer buffer solution 20ml, subsequently, by the flow rate of 4ml/m, the second perfusate of the following presentation made into the temperature of 4 degrees C was poured into the hepatic portal vein, and carried out perfusion for 5 minutes.
Dimethyl sulfoxide 2gD-mannite 1 gKrebs-Ringer buffer solution 20ml [0026] (3) Liver was extracted immediately after cold storage perfusion, and the metal needle aggregate was run through. After storing this in the form polystyrene heat insulation box, it stored all over the -80-degree C freezer (slowly cooled at less than 1 degree C/m). The heat insulation box was filled with the liquid nitrogen of optimum dose after 3-hour or more progress, and it cooled further. The liver sample was saved for three weeks, filling up liquid nitrogen.
[0027] (4) The wave plate was fixed to the location with a depth [of a constant temperature bath with a defrosting temperature / of an organization / of 25 degrees C] of 2 millimeters. The liver sample which it let pass to the metal needle was taken out from the heat insulation box as it was, and it carried on this wave plate. the 25-degree C constant temperature to which a metal plinth flows around -- it is heated with water. It took out after 2 minutes, the liver sample was extruded using the grid of the glass rod inserted in the plinth side of a metal needle, and it removed from the needle. Perfusion of the first perfusate with a temperature of 4 degrees C was poured in and carried out to liver for 5 minutes by the flow rate of 4ml/m from the portal vein the bottom picking outside, and the second perfusate in liver was permuted by the first perfusate.
[0028] (5) After carrying out formalin fixation of the check liver sample of organization preservation, it dyed by making it an intercept with a thickness of 5 microns. As a result of microscope observation, the cell of liver is saved well and especially the glycogen granule was accepted clearly.
[0029] [Example 2]
(1) organization extraction and perfusion -- the abdominal cavity of a healthy rat was injected with 0.1ml of chloro bromazine 1% water solutions, and the weight of 100g, the abdominal peripheral nerve was intercepted, after injecting with and anesthetizing 0.04mg [per weight of 100g] SOMUNO pentyl, the laparotomy was performed and the detention needle connected with the polyethylene capillary was inserted in the hepatic portal vein. According to the conventional method of liver perfusion, it is per minute about the first perfusate of the following presentation which made temperature -5 degrees C from the polyethylene capillary connected with the detention needle. By the flow rate of 0.9ml, it poured into the hepatic portal vein and perfusion was carried out for 4 minutes. while carrying out perfusion -- liver -- the law from a rat -- it excised by the method.

D-glucose 2 gBSA 4.5 g enzyme inhibitor Pefabloc (Merck) 1 mM equivalent p-APMSF 0.1 mM equivalent (p-amidinophenylmethanesulfonylfluoride hydrochloride) Krebs-Henseleit Buffer solution 100 Milliliter (pH 7.4) Chloro bromazine 1 g heparin 0.5 Milliliter [0030] While being immersed into the liquid (the second perfusate) of the following presentation which made extracted liver immediately the temperature of -5 degrees C, it is per minute about the second perfusate. By the flow rate of 0.9ml, it poured into the hepatic portal vein and perfusion was carried out for 4 minutes. Dimethyl sulfoxide 10gD-mannite 5gD-glucose 2 gBSA 4.5g enzyme inhibitor Pefabloc (Merck) 1 mM equivalent p-APMSF It is Krebs-Henseleit by 0.1mM. Buffer solution 100 Milliliter (pH 7.4) [0031] (2) The metal needle aggregate (diameter of a gilding brass needle and a needle 5mm in 0.8mm, main spacing of 3mm, height of 20mm of a needle, thickness of a metal plinth) was run through with liver immediately after cold storage perfusion. The base of a metal needle is beforehand stabbed with the synthetic paper. This was enclosed with the cold-resistance polyethylene bag, and it cooled to -80 degrees C all over the program control freezer set as -1 degree C per minute. After 3-hour or more progress, in the heat insulation box, the liquid nitrogen of optimum dose was poured out and it cooled further. The liver sample was saved for three weeks, filling up liquid nitrogen.

[0032] (3) After taking out the container of a sample out of the defrosting liquid nitrogen of an organization and placing for a while all over a -80-degree C freezer, in the case of 50g organization weight, the temperature of the whole sample was raised to -4 degrees C in a short time for less than 2 minutes by circulating liquid in the defrosting liquid of the same presentation as the first perfusate.

[0033] The sample was sampled from the metal needle aggregate in defrosting liquid, perfusion of the first perfusate with a temperature of -4 degrees C was poured in and carried out from the detention needle for 4 minutes by the flow rate of 1ml/m to the portal vein, and the second perfusate in liver was permuted by the first perfusate.

[0034] When some organizations were fixed and dyed with the conventional method and optical microscope observation was carried out, most change of an organization was not seen as compared with the organization immediately after excision (only perfusion by the first perfusate).

[0035] [Example 3]

(1) preparation of perfusion -- the abdominal cavity of a healthy rat was injected with the 0.1ml [per weight of 100g] chlorpromazine 1% water solution, the abdominal peripheral nerve was intercepted, per ml, after carrying out 50 microliter injection per weight of 100g, halothane anesthesia of the heparin liquid of 1000IU was carried out with the conventional method, the laparotomy was performed, and the detention needle connected with the polyethylene capillary was inserted in the hepatic portal vein (portal vein cannulation). Cannulation was performed also to the bottom main artery by the same approach (bottom main artery cannulation), and the bottom main artery and the inferior vena cava were litigated collectively. The main artery was stopped by the crane mel, the thorax was opened and a main artery and vena cava were cut.

[0036] (2) Cut the ligation section of a perfusion inferior vena cava, lead bottom main artery cannulation in the first perfusate of the following presentation which made temperature -5 degrees C, and it is per minute. Portal vein cannulation is led to a bottom main artery by the flow rate of 1.5ml, and it is per minute. By the flow rate of 1.0ml, it

poured into the hepatic portal vein, respectively, and perfusion was carried out for 10 minutes. After checking that blood had been enough eliminated from liver, liver was extracted from the rat. At this time, a portal vein and a bottom main artery secure each cannulation to a liver side, and are cut.
The first perfusate: D-glucose 2 gBSA 3 gKrebs-Henseleit Buffer solution 100 Milliliter (pH 7.4) Chlorpromazine 0.01g [0037] While being immersed into the liquid (the second perfusate) of the following presentation which made extracted liver immediately the temperature of -5 degrees C, bottom main artery cannulation is led in this second perfusate, and it is per minute. Portal vein cannulation is led to a bottom main artery by the flow rate of 1.5ml, and it is per minute. By the flow rate of 1.0ml, it poured into the hepatic portal vein, respectively, and perfusion was carried out for 10 minutes.
Dimethyl sulfoxide 10 gD-mannite 5 gBSA 3 gKrebs-Henseleit Buffer solution 100 Milliliter (pH 7.4) [0038] (2) The metal needle aggregate (diameter of a gilding brass needle and a needle 5mm in 0.8mm, main spacing of 3mm, height of 20mm of a needle, thickness of a metal plinth) was run through with liver immediately after cold storage perfusion. The base of a metal needle is beforehand stabbed with the synthetic paper. This was enclosed with the cold-resistance polyethylene bag, and it cooled to -80 degrees C all over the program control freezer set as -1 degree C per minute. After 3-hour or more progress, in the heat insulation box, the liquid nitrogen of optimum dose was poured out and it cooled further. The liver sample was saved for three weeks, filling up liquid nitrogen.
[0039] (3) After taking out the container of a sample out of the defrosting liquid nitrogen of an organization and placing for a while all over a -80-degree C freezer, the organization sample was dipped in the defrosting liquid (temperature of about 30 degrees C) of the same presentation as the first perfusate, and, in the case of 50g organization weight, the temperature of the whole sample was raised to 4 degrees C in a short time for less than 2 minutes by circulating liquid.
[0033] A sample is sampled from the metal needle aggregate in defrosting liquid, bottom main artery cannulation is led in the third perfusate of the following presentation, and it is per minute. Portal vein cannulation is led to a bottom main artery by the flow rate of 1.5ml, and it is per minute. By the flow rate of 1.0ml, it poured into the hepatic portal vein, respectively, perfusion was carried out for 10 minutes, and the second perfusate in liver was permuted by the third perfusate. Temperature of the third perfusate was made into 4 degrees C at first, and raised temperature to 25 degrees C at a rate of about 2.5 degrees C per minute in perfusion.
The third perfusate: D-mannitol 1 gD-glucose 1 g lactobionate 50 mMBSA 3 g ascorbic acid 0.1 gKrebs-Henseleit Buffer solution 100 Milliliter (pH 7.4) [0040] When some organizations were fixed and dyed with the conventional method and optical microscope observation was carried out, most change of an organization was not seen as compared with the organization immediately after excision (only perfusion by the first perfusate).
[0041] They are the temperature of 37 degrees C, and relative humidity about the liver which finished perfusion by the third perfusate in the example 3. It stores into 100% of box, and is [2-14C] of 185kBq. Diazepam was poured in from portal vein cannulation and the perfusate for a metabolic turnover experiment of the following presentation was supplied by the flow rate of 1 ml/min using the immediately after metering pump. This liquid was supplied to coincidence by the flow rate of 1.5 ml/min through artery

cannulation. Perfusion was continued for 20 minutes, the perfusate which flows out from liver at this time was received in ROUTO, and it extracted in the test tube every 2 minutes.

[0042] The extracted perfusate carried out at-long-intervals alignment separation by 3000 rotations for 10 minutes, separated the radioactive metabolite in the supernatant liquid by thin-layer chromatography, and performed detection analysis by radio RUMINOGURAFI. Consequently, 4'-hydroxydiazepam and Nordazepam which are the metabolite besides Diazepam which is a parent compound in effluent, Temazepam, and Oxazepam It was detected. These are metabolite accepted when the same experiment is conducted using the liver which does not carry out cryopreservation, and show that the liver tissue saved by this approach has metabolic activity.

[0043] Moreover, any effluent is abbreviation when the quantum of the lactate dehydrogenase (LDH) was carried out. It is 100IU / liter and normal values were shown through the perfusion for 20 minutes. This shows that liver tissue has not received damage even if it passes through the process of freezing and defrosting.